80406 Access DB# _____

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SEARCH REQUEST FORM

CRF

Scientific and Technical Information Center

Requester's Full Name: Khatol Shahnan-Shah Examiner #: 78586 Date: 11/15/02

Art Unit: 1644 Phone Number 30 8-8096 Serial Number: 09/848,791

Mail Box and Bldg/Room Location: 8D-16 Results Format Preferred (circle): PAPER DISK E-MAIL

8E-16

If more than one search is submitted, please prioritize searches in order of need.

****

Please provide a detailed statement of the search topic, and describe as specifically as possible the subject matter to be searched. Include the elected species or structures, keywords, synonyms, acronyms, and registry numbers, and combine with the concept or utility of the invention. Define any terms that may have a special meaning. Give examples or relevant citations, authors, etc, if known. Please attach a copy of the cover sheet, pertinent claims, and abstract.

Title of Invention: _____

Inventors (please provide full names): See attached bib Sheet

Earliest Priority Filing Date: _____

**For Sequence Searches Only* Please include all pertinent information (parent, child, divisional, or issued patent numbers) along with the appropriate serial number.*

Point of Contact:
Beverly Shears
Technical Info. Specialist
CM1 1E05 Tel: 308-4994

Please search

Seq IDs # 1-4

Sequence list attached

Please search claims.

1-9

Keywords highlighted.

Copy of Claims and Abstract attached.

AA1, 9
2, 11
AA4-30
3-11 Thanks.

****

STAFF USE ONLY

Searcher: Beverly c 4994

Searcher Phone #: _____

Searcher Location: _____

Date Searcher Picked Up: _____

Date Completed: 11-25-02

Searcher Prep & Review Time: 20

Clerical Prep Time: _____

Online Time: 29

Type of Search

NA Sequence (#) _____

AA Sequence (#) _____

Structure (#) _____

Bibliographic _____

Litigation _____

Fulltext _____

Patent Family _____

Other _____

Vendors and cost where applicable

STN ✓

Dialog _____

Questel/Orbit _____

Dr.Link _____

Lexis/Nexis _____

Sequence Systems _____

WWW/Internet _____

Other (specify) CGN

PTO-1590 (8-01)

According to the Pre Publication Rules, every patent application received by the United States Patent and Trademark Office after November 29, 2000 will be pre-published at eighteen months from the effective filing date. When the application is published the contents, including the sequences, will become prior art.

Two new databases have been created to hold the pre-published sequences:
Published_Applications_NA contains nucleic acid sequences; the search results will have the extension **.rnpb**.
Published_Applications_AA contains amino acid sequences; the search results will have the extension **.rapb**.

Each pre-published application is given a unique Publication Number. An example of a Publication Number is US20021234567A1. The "US" indicates the application was a U.S. application. The first 4 digits show the calendar year the application was published. The next 7 digits represent when the application was published. This 7-digit number starts at zero at the beginning of each calendar year. Each application published is given the next number in order. The "A" indicates a utility patent application and the "1" shows that this was the first time the application had been published. If the applicants submit changes to the application, they may requests that the changed application be published again. In such instances, the "1" at the end of the number would be replaced by a "2".

Sequences in the PGPub database are public information; it is permissible to leave these results in the case.

Shahnan-Shah
09/848781

FILE 'REGISTRY' ENTERED AT 14:12:32 ON 25 NOV 2002
E PROTEASE/CN 5
L1 1 SEA ABB=ON PLU=ON PROTEASE/CN
E PROTEINASE/CN 5
L2 3568 SEA ABB=ON PLU=ON PROTEINASE ?/CN
L3 3568 SEA ABB=ON PLU=ON L1 OR L2

-Key terms

FILE 'HCAPLUS' ENTERED AT 14:13:09 ON 25 NOV 2002
L4 25 SEA ABB=ON PLU=ON (L3 OR PROTEASE OR PROTEINASE) AND (PRO!ARYOT?(S)(MICROB## OR PATHOGEN OR MICROORGAN? OR MICRO ORGAN? OR BACTERI## OR MONOCYTOGENES))

L4 ANSWER 1 OF 25 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2002:736712 HCAPLUS
DOCUMENT NUMBER: 137:262206
TITLE: Recombinant bacterial phytases and their use for degradation of food phytates.
INVENTOR(S): Short, Jay M.; Kretz, Keith A.; Gray, Kevin A.; Barton, Nelson Robert; Garrett, James B.; O'Donoghue, Eileen; Mathur, Eric J.
PATENT ASSIGNEE(S): USA
SOURCE: U.S. Pat. Appl. Publ., 62 pp., Cont.-in-part of U.S. Ser. No. 580,515.
CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 5
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2002136754	A1	20020926	US 2001-866379	20010524
US 5876997	A	19990302	US 1997-910798	19970813
US 6110719	A	20000829	US 1999-259214	19990301
US 6190897	B1	20010220	US 1999-291931	19990413
US 6183740	B1	20010206	US 1999-318528	19990525

PRIORITY APPLN. INFO.:
US 1997-910798 A3 19970813
US 1999-259214 A2 19990301
US 1999-291931 A1 19990413
US 1999-318528 A2 19990525
US 2000-580515 A2 20000525

AB A purified and modified phytase enzyme from Escherichia coli K12 appA phytase is provided. The enzyme has phytase activity and improved thermal tolerance as compared with the wild-type enzyme. In addn., the enzyme has improved **protease** stability at low pH. Glycosylation of the modified phytase provided a further improved enzyme having improved thermal tolerance and **protease** stability. The enzyme can be produced from native or recombinant host cells and can be used to aid in the digestion of phytate where desired. In particular, the phytase of the present invention can be used in foods to improve the nutritional value of phytate rich ingredients.

L4 ANSWER 2 OF 25 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2002:576445 HCAPLUS
DOCUMENT NUMBER: 137:274925
TITLE: Sequence conservation in the chagasin family suggests a common trend in cysteine

proteinase binding by unrelated protein inhibitors

AUTHOR(S): Rigden, Daniel J.; Mosolov, Vladimir V.; Galperin, Michael Y.

CORPORATE SOURCE: National Centre of Genetic Resources and Biotechnology, Cenargen/Embrapa, S.A.I.N. Parque Rural, Brasilia, 70770-900, Brazil

SOURCE: Protein Science (2002), 11(8), 1971-1977
CODEN: PRCIEI; ISSN: 0961-8368

PUBLISHER: Cold Spring Harbor Laboratory Press

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The recently described inhibitor of cysteine **proteinases** from Trypanosoma cruzi, chagasin, was found to have close homologs in several eukaryotes, **bacteria** and archaea, the first protein inhibitors of cysteine **proteases** in **prokaryotes**. These previously uncharacterized 110-130 residue-long proteins share a well-conserved sequence motif that corresponds to two adjacent .beta.-strands and the short loop connecting them. Chagasin-like proteins also have other conserved, mostly arom., residues, and share the same predicted secondary structure. These proteins adopt an all-.beta. fold with eight predicted .beta.-strands of the Ig type. The phylogenetic distribution of the chagasins generally correlates with the presence of papain-like cysteine **proteases**. Previous studies have uncovered similar trends in cysteine **proteinase** binding by two unrelated inhibitors, stefin and p41, that belong to the cystatin and thyroglobulin families, resp. A hypothetical model of chagasin-cruzipain interaction suggests that chagasin may dock to the cruzipain active site in a similar manner with the conserved NPTTG motif of chagasin forming a loop that is similar to the wedge structures formed at the active sites of papain and cathepsin L by stefin and p41.

REFERENCE COUNT: 56 THERE ARE 56 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 3 OF 25 HCAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 2002:107584 HCAPLUS

DOCUMENT NUMBER: 136:131210

TITLE: A device for detecting bacterial contamination and method of use

INVENTOR(S): Sanders, Mitchell C.

PATENT ASSIGNEE(S): Expressive Constructs, Inc., USA

SOURCE: PCT Int. Appl., 25 pp.
CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002010433	A2	20020207	WO 2001-US14613	20010503

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO,

NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: US 2000-201405P P 20000503

AB A device and method for detecting the presence or absence of a **prokaryotic microorganism** are provided, comprising the steps of identifying a protein, such as a microbial-specific **protease** that characterizes the presence of a specific **prokaryotic microbe** and thereby provides a marker for that **microbe**; detecting the **protease** that is a marker for the presence of a specific **prokaryotic microbe** by cleaving a substance when the **protease** is present; and signaling the presence of that **protease** when cleavage has occurred. More specifically, the method comprises identifying at least one outer membrane protein or a secreted protein that is unique to a particular microbial pathogen such as for example Listeria monocytogenes and that is substrate specific.

IT **9001-92-7, Protease**
RL: ANT (Analyte); BSU (Biological study, unclassified); ANST (Analytical study); BIOL (Biological study)
(Microbial-specific; device for detecting bacterial contamination and method of use)

L4 ANSWER 4 OF 25 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2002:51536 HCAPLUS
DOCUMENT NUMBER: 136:113828
TITLE: Production of glucan-like polysaccharide by exopolysaccharide-producing bacterial strains and sequences of wss and wsp operon from Pseudomonas
INVENTOR(S): Rainey, Paul Barton; Spiers, Andrew Julien; Bantinaki, Eleni
PATENT ASSIGNEE(S): Isis Innovation Limited, UK
SOURCE: PCT Int. Appl., 186 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002004526	A2	20020117	WO 2001-GB3077	20010709

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

AU 2001069311 A5 20020121 AU 2001-69311 20010709
PRIORITY APPLN. INFO.: GB 2000-16842 A 20000707
WO 2001-GB3077 W 20010709

AB The invention is concerned with the identification of a novel class of bacterial polysaccharide biosynthetic operons and an ovel class of regulatory operons involved with polysaccharide biosynthesis, bacterial attachment and biofilm development. Bacterial strains which possess a polysaccharide biosynthetic operon of the type provide by the invention are capable of producing polysaccharide with industrial implications. Bacterial strains which possess a regulatory operon of the type provided by the invention may be targeted by pharmaceutical/chem. agents to prevent bacterial attachment and biofilm development.

IT **39450-01-6**
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(used in cell lysis; prodn. of glucan-like polysaccharide by exopolysaccharide-producing bacterial strains and sequences of wss and wsp operon from Pseudomonas)

L4 ANSWER 5 OF 25 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2001:776072 HCAPLUS
DOCUMENT NUMBER: 136:34384
TITLE: Horizontal gene transfer in prokaryotes: Quantification and classification
AUTHOR(S): Koonin, Eugene V.; Makarova, Kira S.; Aravind, L.
CORPORATE SOURCE: National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health, Bethesda, MD, 20894, USA
SOURCE: Annual Review of Microbiology (2001), 55, 709-742
CODEN: ARMIAZ; ISSN: 0066-4227
PUBLISHER: Annual Reviews Inc.
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English

AB A review. Comparative anal. of **bacterial**, archaeal, and eukaryotic genomes indicates that a significant fraction of the genes in the **prokaryotic** genomes have been subject to horizontal transfer. In some cases, the amt. and source of horizontal gene transfer can be linked to an organism's lifestyle. For example, bacterial hyperthermophiles seem to have exchanged genes with archaea to a greater extent than other bacteria, whereas transfer of certain classes of eukaryotic genes is most common in parasitic and symbiotic bacteria. Horizontal transfer events can be classified into distinct categories of acquisition of new genes, acquisition of paralogs of existing genes, and xenologous gene displacement whereby a gene is displaced by a horizontally transferred ortholog from another lineage (xenolog). Each of these types of horizontal gene transfer is common among prokaryotes, but their relative contributions differ in different lineages. The fixation and long-term persistence of horizontally transferred genes suggests that they confer a selective advantage on the recipient organism. In most cases, the nature of this advantage remains unclear, but detailed examn. of several cases of acquisition of eukaryotic genes by bacteria seems to reveal the evolutionary forces involved. Examples include isoleucyl-tRNA synthetases whose acquisition from eukaryotes by several bacteria is linked to

antibiotic resistance, ATP/ADP translocases acquired by intracellular parasitic bacteria, Chlamydia and Rickettsia, apparently from plants, and **proteases** that may be implicated in chlamydial pathogenesis.

REFERENCE COUNT: 119 THERE ARE 119 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 6 OF 25 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2001:576748 HCAPLUS
DOCUMENT NUMBER: 135:238553
TITLE: Membrane topology of the Streptomyces lividans type I signal peptidases
AUTHOR(S): Geukens, Nick; Lammertyn, Elke; Van Mellaert, Lieve; Schacht, Sabine; Schaerlaekens, Kristien; Parro, Victor; Bron, Sierd; Engelborghs, Yves; Mellado, Rafael P.; Anne, Jozef
CORPORATE SOURCE: Laboratory of Bacteriology, Rega Institute, Katholieke Universiteit Leuven, Louvain, 3000, Belg.
SOURCE: Journal of Bacteriology (2001), 183(16), 4752-4760
CODEN: JOBAAY; ISSN: 0021-9193
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Most bacterial membranes contain one or two type I signal peptidases (SPases) for the removal of signal peptides from export proteins. For Streptomyces lividans, four different type I SPases (denoted SipW, SipX, SipY, and SipZ) were previously described. In this communication, we report the exptl. detn. of the membrane topol. of these SPases. A **protease** protection assay of SPase tendamistat fusions confirmed the presence of the N- as well as the C-terminal transmembrane anchor for SipY. SipX and SipZ have a predicted topol. similar to that of SipY. These three S. lividans SPases are currently the only known **prokaryotic**-type type I SPases of gram-pos. **bacteria** with a C-terminal transmembrane anchor, thereby establishing a new subclass of type I SPases. In contrast, S. lividans SipW contains only the N-terminal transmembrane segment, similar to most type I SPases of gram-pos. bacteria. Functional anal. showed that the C-terminal transmembrane anchor of SipY is important to enhance the processing activity, both in vitro as well as in vivo. Moreover, for the S. lividans SPases, a relation seems to exist between the presence or absence of the C-terminal anchor and the relative contributions to the total SPase processing activity in the cell. SipY and SipZ, two SPases with a C-terminal anchor, were shown to be of major importance to the cell. Accordingly, for SipW, missing the C-terminal anchor, a minor role in preprotein processing was found.

REFERENCE COUNT: 49 THERE ARE 49 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 7 OF 25 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2001:402295 HCAPLUS
DOCUMENT NUMBER: 135:235893
TITLE: Identification of novel potent hydroxamic acid inhibitors of peptidyl deformylase and the

importance of the hydroxamic acid functionality on inhibition

AUTHOR(S): Thorarensen, A.; Douglas, M. R., Jr.; Rohrer, D. C.; Vosters, A. F.; Yem, A. W.; Marshall, V. D.; Lynn, J. C.; Bohanon, M. J.; Tomich, P. K.; Zurenko, G. E.; Sweeney, M. T.; Jensen, R. M.; Nielsen, J. W.; Seest, E. P.; Dolak, L. A.

CORPORATE SOURCE: Medicinal Chemistry 7254-209-615, Pharmacia, Kalamazoo, MI, 49001-019, USA

SOURCE: Bioorganic & Medicinal Chemistry Letters (2001), 11(11), 1355-1358
CODEN: BMCLE8; ISSN: 0960-894X

PUBLISHER: Elsevier Science Ltd.

DOCUMENT TYPE: Journal

LANGUAGE: English

OTHER SOURCE(S): CASREACT 135:235893

AB Peptidyl deformylase (PDF) is a metallo **protease** that catalyzes the removal of a formyl group from the N-termini of **prokaryotic** prepd. polypeptides, an essential step in **bacterial** protein synthesis. Screening of our compd. collection using Staphylococcus aureus PDF afforded a very potent inhibitor with an IC50 in the low nanomolar range. Unfortunately, the compd. that contains a hydroxamic acid did not exhibit antibacterial activity (MIC). In order to address the lack of activity in the MIC assay and to det. what portion of the mol. was responsible for binding to PDF, we prepd. several analogs. This paper describes our findings that the hydroxamic acid functionality is mainly responsible for the high affinity to PDF. In addn., we identified an alternative class of PDF inhibitors which has both PDF and antibacterial activity. The discovery of PDF inhibitors and evaluation of various metal coordination groups on PDF activity is reported.

REFERENCE COUNT: 26 THERE ARE 26 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 8 OF 25 HCAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 2001:265254 HCAPLUS

DOCUMENT NUMBER: 134:275746

TITLE: Gallium complexes of 3-hydroxy-4-pyrones to treat infection by intracellular prokaryotes, DNA viruses and retroviruses

INVENTOR(S): Bernstein, Lawrence R.

PATENT ASSIGNEE(S): USA

SOURCE: PCT Int. Appl., 46 pp.
CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 2

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001024799	A1	20010412	WO 2000-US28174	20001004

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ,

PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG
EP 1218011 A1 20020703 EP 2000-973473 20001004
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL
PRIORITY APPLN. INFO.: US 1999-157460P P 19991004
WO 2000-US28174 W 20001004

AB Methods are provided for treating or preventing infections by obligate intracellular prokaryotes, including mycoplasma, rickettsia and chlamydia, retroviruses, and DNA viruses, including herpesviruses, papillomaviruses, adenoviruses and hepatitis B virus. Emphasis is placed on providing methods for the treatment of HIV disease. In addn. to providing methods for treating HIV infection itself, methods are provided for treating an HIV patient having a co-infection by another retrovirus, an obligate intracellular prokaryote, or a DNA virus. The methods involve the administration of 3:1 complexes of 3-hydroxy-4-pyrones with gallium, e.g., gallium maltolate. Therapies incorporating gallium maltolate in combination with agents used against obligate intracellular **prokaryote**, retrovirus and DNA virus **pathogens** are also provided, as are multicombination therapies designed to treat co-infection by an obligate intracellular **prokaryote**, retrovirus or DNA virus in an individual infected by HIV. These multi-combination therapies rely on the ability of gallium maltolate to complement antiviral medication regimes against both HIV and other pathogens such as herpesvirus infections, including Kaposi sarcoma, CMV retinitis and blindness, and lymphomas, in patients immunocompromised by HIV infection.

IT **9001-92-7, Protease**
RL: BSU (Biological study, unclassified); BIOL (Biological study) (inhibitors; hydroxypyrone-gallium complexes to treat infection by intracellular prokaryotes, DNA viruses, and retroviruses, and use with other agents)

REFERENCE COUNT: 2 THERE ARE 2 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 9 OF 25 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2001:132042 HCAPLUS
DOCUMENT NUMBER: 134:248643
TITLE: Proteasomes in prokaryotes
AUTHOR(S): Zwickl, Peter; Goldberg, Alfred L.; Baumeister, Wolfgang
CORPORATE SOURCE: Molekulare Strukturbiologie Max-Planck-Institut fur Biochemie, Martinsried, Germany
SOURCE: Molecular Biology Intelligence Unit (2000), 12(Proteasomes: The World of Regulatory Proteolysis), 8-20
CODEN: MBIUF8; ISSN: 1431-0414
PUBLISHER: R. G. Landes Co.
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English

AB A review, with 78 refs. The topics discussed include: occurrence of proteasomes in archaea and **bacteria**; subunit compn. of

proteasomes; the structure, mechanism, and assembly of **prokaryotic** proteasomes; the HsIVU **protease** complex; evolution of proteasomal subunits; ATP-dependent proteolysis in archaea; evolution of regulatory complexes; and functions and redundancy of proteolytic systems in **prokaryotes**.

REFERENCE COUNT: 79 THERE ARE 79 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 10 OF 25 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2000:845581 HCAPLUS
DOCUMENT NUMBER: 134:127339
TITLE: Programmed cell death
AUTHOR(S): Samuilov, V. D.; Oleskin, A. V.; Lagunova, E. M.
CORPORATE SOURCE: Department of Cell Physiology and Immunology, School of Biology, Lomonosov Moscow State University, Moscow, 119899, Russia
SOURCE: Biochemistry (Moscow)(Translation of Biokhimiya (Moscow)) (2000), 65(8), 873-887
CODEN: BIORAK; ISSN: 0006-2979
PUBLISHER: MAIK Nauka/Interperiodica Publishing
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English
AB A review, with 135 refs., on programmed cell death (apoptosis) in animals and plants. Necrosis is a pathol. scenario of cell death, which entails an inflammatory response in animal tissues. Apoptosis results in the disintegration of animal/plant cells into membrane vesicles enclosing the intracellular content, which are thereupon engulfed by adjacent or specialized cells (phagocytes) in animals. Plants lack such specialized cells, and plant cell walls prevent phagocytosis. The paper considers the main mol. mechanisms of apoptosis in animals and the pathways of activation of caspases, evolutionarily conserved cysteine **proteases**. A self-contained section concerns itself with the process of programmed cell death (PCD) in **microorganisms** including: (1) cell death in the myxomycete Dictyostelium discoideum and the parasitic flagellate Trypanosoma cruzi; (2) PCD in genetically manipulated yeast expressing the proapoptotic Bax and Bak proteins; (3) the death of a part of a **prokaryotic** cell population upon the depletion of nutrient resources or under stress; (4) the elimination of cells after a loss of a plasmid encoding a stable cytotoxic agent in combination with an unstable antidote; and (5) PCD in phage-infected **bacterial** cells.
REFERENCE COUNT: 135 THERE ARE 135 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 11 OF 25 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2000:243779 HCAPLUS
DOCUMENT NUMBER: 133:14052
TITLE: Role of Ser-652 and Lys-692 in the **protease** activity of infectious bursal disease virus VP4 and identification of its substrate cleavage sites
AUTHOR(S): Lejal, Nathalie; Da Costa, Bruno; Huet, Jean-Claude; Delmas, Bernard
CORPORATE SOURCE: Unite de Virologie et Immunologie moleculaires,

Institut National de la Recherche Agronomique, Jouy-en-Josas, F-78350, Fr.
SOURCE: Journal of General Virology (2000), 81(4), 983-992
CODEN: JGVIAY; ISSN: 0022-1317
PUBLISHER: Society for General Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The polyprotein of infectious bursal disease virus (IBDV), an avian birnavirus, is processed by the viral **protease**, VP4. Previous data obtained on the VP4 of infectious pancreatic necrosis virus (IPNV), a fish birnavirus, and comparative sequence anal. between IBDV and IPNV suggest that VP4 is an unusual eukaryotic serine **protease** that shares properties with **prokaryotic** leader peptidases and other **bacterial** peptidases. IBDV VP4 is predicted to utilize a serine-lysine catalytic dyad. Replacement of the members of the predicted catalytic dyad (Ser-652 and Lys-692) confirmed their indispensability. The two cleavage sites at the pVP2-VP4 and VP4-VP3 junctions were identified by N-terminal sequencing and probed by site-directed mutagenesis. Several addnl. candidate cleavage sites were identified in the C-terminal domain of pVP2 and tested by cumulative site-directed mutagenesis and expression of the mutant polyproteins. The results suggest that VP4 cleaves multiple (Thr/Ala)-X-Ala Ala motifs. A trans activity of the VP4 **protease** of IBDV, and also IPNV VP4 **protease**, was demonstrated by co-expression of VP4 and a polypeptide substrate in Escherichia coli. For both **proteases**, cleavage specificity was identical in the cis- and trans-activity assays. An attempt was made to det. whether VP4 **proteases** of IBDV and IPNV were able to cleave heterologous substrates. In each case, no cleavage was obsd. with heterologous combinations. These results on the IBDV VP4 confirm and extend our previous characterization of the IPNV VP4, delineating the birnavirus **protease** as a new type of viral serine **protease**.

IT **37259-58-8**, Serine **proteinase**
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(VP4; role of Ser-652 and Lys-692 in the **protease** activity of infectious bursal disease virus VP4 and identification of its substrate cleavage sites)

REFERENCE COUNT: 24 THERE ARE 24 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 12 OF 25 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1999:692558 HCAPLUS
DOCUMENT NUMBER: 132:175178
TITLE: Mechanism of action of anti-fungal drugs
AUTHOR(S): Nakashima, Shigeru
CORPORATE SOURCE: Department of Biochemistry, School of Medicine, Gifu University, Japan
SOURCE: Nippon Ishinkin Gakkai Zasshi (1999), 40(3), 119-123
CODEN: NIGZE4; ISSN: 0916-4804
PUBLISHER: Nippon Ishinkin Gakkai
DOCUMENT TYPE: Journal; General Review

LANGUAGE: Japanese

AB A review and discussion with 26 refs. Clin. application of recently developed anti-fungal drugs including fluconazole and itraconazole has provided great advantages in the treatment of deep mycoses. However, pathogenic fungi belong to eukaryotes including humans and are phylogenetically apart from **prokaryotes**, i.e. **bacteria**. In other words, the components and metabolic pathways of fungi and mammals are very similar. This sometimes makes it difficult to treat severe mycoses with the anti-fungal drugs available at present. Therefore, new drugs which are more selective for fungal components, such as new azoles, are desired by clinicians and some of them are now under clin. trial. Fungal factors involved in dimorphic change including transcription factors and members of MAP kinase cascades as well as virulence factors including **proteases**, phospholipases and catalase have recently been identified. These factors and enzymes responsible for cell wall construction could be selective targets to develop new anti-fungal drugs.

L4 ANSWER 13 OF 25 HCAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1999:492859 HCAPLUS

DOCUMENT NUMBER: 132:34291

TITLE: .alpha.2-macroglobulin: an evolutionarily conserved arm of the innate immune system

AUTHOR(S): Armstrong, Peter B.; Quigley, James P.

CORPORATE SOURCE: Marine Biological Laboratory, Woods Hole, MA, 02543, USA

SOURCE: Developmental & Comparative Immunology (1999), 23(4-5), 375-390
CODEN: DCIMDQ; ISSN: 0145-305X

PUBLISHER: Elsevier Science Ltd.

DOCUMENT TYPE: Journal; General Review

LANGUAGE: English

AB A review with 95 refs. All animals and plants have immune systems that protect them from the diversity of pathogens that would otherwise threaten their survival. The different components of the immune system may inactivate the pathogens themselves or promote the inactivation and clearance of toxic products produced by the pathogens. An important category of virulence factors of **bacterial** and **prokaryotic pathogens** are the **proteases**, which act to facilitate the invasion of the **pathogens** and to promote their destructive growth in the host organism. The present review concs. on the comparative biol. of an evolutionarily conserved arm of the immune system, the protein, .alpha.2-macroglobulin. .alpha.2-Macroglobulin is an abundant protein of the plasma of vertebrates and members of several invertebrate phyla and functions as a broad-spectrum **protease**-binding protein. **Protease**-conjugated .alpha.2-macroglobulin is selectively bound by cells contacting the body fluids and .alpha.2-macroglobulin and its **protease** cargo are then internalized and degraded in secondary lysosomes of those cells. In addn. to this function as an agent for **protease** clearance, .alpha.2-macroglobulin binds a variety of other ligands, including several peptide growth factors and modulates the activity of a lectin-dependent cytolytic pathway in arthropods.

REFERENCE COUNT: 95 THERE ARE 95 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE

IN THE RE FORMAT

L4 ANSWER 14 OF 25 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1999:172633 HCAPLUS
DOCUMENT NUMBER: 130:220164
TITLE: Rapid detection and identification of microorganisms by cell wall or membrane degradation and reaction with probes
INVENTOR(S): Schut, Frederik; Tan, Paris Som Twan
PATENT ASSIGNEE(S): Microscreen B.V., Neth.
SOURCE: PCT Int. Appl., 70 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9910533	A1	19990304	WO 1998-NL481	19980826
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
AU 9888904	A1	19990316	AU 1998-88904	19980826
EP 1009862	A1	20000621	EP 1998-940684	19980826
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				

PRIORITY APPLN. INFO.: EP 1997-202618 A 19970826
WO 1998-NL481 W 19980826

AB The invention relates to the field microbiol., more specifically to the field of detection, identification and quantification or enumeration of microorganisms. **Microorganisms**, such as viruses, plasmids, **bacteria**, yeasts, fungi, algae, protozoa, plant or animal cells, and other **prokaryotic** or eukaryotic cells are in general unicellular organisms with dimensions beneath the limits of vision which thus escape easy detection. The invention provides methods and means for use in situ staining of microorganisms comprising: a) mixing a material contg. at least one microorganism with a compn. which can (partly) degrade a cell wall or cell membrane of a microorganism thereby allowing for penetration through said wall and/or membrane of a (labeled) probe into said microorganism, b) fixing said microorganism with a fixative to retain its individual corpuscular character, c) reacting said probe with an antigen or nucleic acid mol. present in said microorganism and d) detecting the presence of said probe in said microorganism. Lactococcus lactis cremoris cells were treated with cell wall-degrading reagent contg. Tris-HCl, pH 7.0, Na taurocholate, CaCl2, sucrose, lysozyme, pancreatic lipase, and finizym and then fixed with paraformaldehyde. The fixed cells were hybridized with horseradish peroxidase-labeled oligonucleotide probe and the probe was detected through HRP-catalyzed reporter deposition using fluoresceine-tyramide substrate and flow cytometry or

epifluorescence microscopy.

IT **9001-92-7, Proteinase 39450-01-6**
RL: ARU (Analytical role, unclassified); BPR (Biological process); BSU (Biological study, unclassified); CAT (Catalyst use); ANST (Analytical study); BIOL (Biological study); PROC (Process); USES (Uses)
(cell wall- or cell membrane-degrading compn. contg.; rapid detection and identification of microorganisms by cell wall or membrane degrdn. and reaction with probes)

REFERENCE COUNT: 9 THERE ARE 9 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 15 OF 25 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1999:109270 HCAPLUS
DOCUMENT NUMBER: 130:307436
TITLE: Toxin-antitoxin systems homologous with relBE of Escherichia coli plasmid P307 are ubiquitous in prokaryotes
AUTHOR(S): Gronlund, Hugo; Gerdes, Kenn
CORPORATE SOURCE: Department of Molecular Biology, Odense University, Odense, DK-5230, Den.
SOURCE: Journal of Molecular Biology (1999), 285(4), 1401-1415
CODEN: JMOBAK; ISSN: 0022-2836
PUBLISHER: Academic Press
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Toxin-antitoxin systems encoded by bacterial plasmids and chromosomes specify two proteins, a cytotoxin and an antitoxin. The antitoxins neutralize the cognate toxins by forming tight complexes with them. The antitoxins are unstable due to degrdn. by cellular **proteases** (Lon or Clp), whereas the toxins are stable. Here we show that orf7 (denoted relBP307) and orf6 (denoted relEP307) of Escherichia coli plasmid P307 are homologous to the relBE genes of E. coli and constitute a two-component toxin-antitoxin system: (1) relEP307 encodes a cytotoxin lethal or inhibitory to host cells; (2) relBP307 encodes an antitoxin that prevents the lethal action of the relE-encoded toxin; (3) RelBP307 antitoxin is degraded by Lon **protease**; (4) RelBP307 antitoxin autoregulates the relBE operon of P307 at the level of transcription; (5) RelEP307 toxin acts as a co-repressor of transcription; and (6) the relBE system stabilizes a mini-P307 replicon by the killing of plasmid-free cells. Using database searching, we found relBE homologs on the chromosomes of many Gram-neg. and Gram-pos. bacteria. Even more surprising, numerous relBE-homologous gene systems are present on the chromosomes of Archaea. Thus, toxin-antitoxin systems homologous with relBE of E. coli are ubiquitous in prokaryotic organisms. (c) 1999 Academic Press.

REFERENCE COUNT: 53 THERE ARE 53 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 16 OF 25 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1999:101687 HCAPLUS
DOCUMENT NUMBER: 130:292948
TITLE: AAA+: A class of chaperone-like ATPases associated with the assembly, operation, and

disassembly of protein complexes

AUTHOR(S): Neuwald, Andrew F.; Aravind, L.; Spouge, John L.; Koonin, Eugene V.

CORPORATE SOURCE: Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 11724, USA

SOURCE: Genome Research (1999), 9(1), 27-43
CODEN: GEREFS; ISSN: 1088-9051

PUBLISHER: Cold Spring Harbor Laboratory Press

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Using a combination of computer methods for iterative database searches and multiple sequence alignment, we show that protein sequences related to the AAA family of ATPases are far more prevalent than reported previously. Among these are regulatory components of Lon and Clp **proteases**, proteins involved in DNA replication, recombination, and restriction (including subunits of the origin recognition complex, replication factor C proteins, MCM DNA-licensing factors and the **bacterial** DnaA, RuvB, and McrB proteins), **prokaryotic** NtrC-related transcription regulators, the Bacillus sporulation protein SpoVJ, Mg2+ and Co2+ chelatases, the Halobacterium GvpN gas vesicle synthesis protein, dynein motor proteins, TorsinA, and Rubisco activase. Alignment of these sequences, in light of the structures of the clamp loader .delta.' subunit of Escherichia coli DNA polymerase III and the hexamerization component of N-ethylmaleimide-sensitive fusion protein, provides structural and mechanistic insights into these proteins, collectively designated the AAA+ class. Whole-genome anal. indicates that this class is ancient and has undergone considerable functional divergence prior to the emergence of the major divisions of life. These proteins often perform chaperone-like functions that assist in the assembly, operation, or disassembly of protein complexes. The hexameric architecture often assocd. with this class can provide a hole through which DNA or RNA remodeling of DNA-protein complexes.

IT **110910-59-3**, Clp **protease**
RL: BSU (Biological study, unclassified); BIOL (Biological study) (regulatory components of, member of AAA+ class of chaperone-like ATPase; AAA+ class of chaperone-like ATPases assocd. with the assembly, operation, and disassembly of protein complexes)

REFERENCE COUNT: 111 THERE ARE 111 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 17 OF 25 HCAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1997:366451 HCAPLUS

DOCUMENT NUMBER: 127:93546

TITLE: Inactivation of Streptococcus pyogenes extracellular cysteine **protease** significantly decreases mouse lethality of serotype M3 and M49 strains

AUTHOR(S): Lukomski, Slawomir; Sreevatsan, Srinand; Amberg, Cornelia; Reichardt, Werner; Wolschnik, Markus; Podbielski, Andreas; Musser, James M.

CORPORATE SOURCE: Section of Molecular Pathobiology, Department of Pathology, Baylor College of Medicine, Houston, TX, 77030, USA

SOURCE: Journal of Clinical Investigation (1997), 99(11), 2574-2580

CODEN: JCINAO; ISSN: 0021-9738
PUBLISHER: Rockefeller University Press
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Cysteine **proteases** have been implicated as important virulence factors in a wide range of **prokaryotic** and eukaryotic **pathogens**, but little direct evidence has been presented to support this notion. Virtually all strains of the human bacterial pathogen Streptococcus pyogenes express a highly conserved extracellular cysteine **protease** known as streptococcal pyrogenic exotoxin B (SpeB). Two sets of isogenic strains deficient in SpeB cysteine **protease** activity were constructed by integrational mutagenesis using nonreplicating recombinant plasmids contg. a truncated segment of the speB gene. Immunoblot analyses and enzyme assays confirmed that the mutant derivs. were deficient in expression of enzymically active SpeB cysteine **protease**. To test the hypothesis that the cysteine **protease** participates in host mortality, the authors assessed the ability of serotype M3 and M49 wild-type strains and isogenic **protease**-neg. mutants to cause death in outbred mice after i.p. inoculation. Compared to wild-type parental organisms, the serotype M3 speB mutant lost virtually all ability to cause mouse death, and similarly, the virulence of the M49 mutant was detrimentally altered. The data unambiguously demonstrate that the streptococcal enzyme is a virulence factor, and thereby provide addnl. evidence that microbial cysteine **proteases** are crit. in host-pathogen interactions.

L4 ANSWER 18 OF 25 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1997:254085 HCAPLUS
DOCUMENT NUMBER: 126:237503
TITLE: Overproduction of human neuronal nitric oxide synthase in prokaryote using coexpression of folding agonists
INVENTOR(S): Masters, Bettie Sue; Roman, Linda J.; Sheta, Essam A.
PATENT ASSIGNEE(S): Board of Regents, the University of Texas System, USA; Masters, Bettie Sue; Roman, Linda J.; Sheta, Essam A.
SOURCE: PCT Int. Appl., 53 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9708299	A1	19970306	WO 1996-US14045	19960823
W: AL, AM, AT, AU, BB, BG, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML				
US 5919682	A	19990706	US 1995-519105	19950824

AU 9669102 A1 19970319 AU 1996-69102 19960823
PRIORITY APPLN. INFO.: US 1995-519105 19950824
WO 1996-US14045 19960823

AB The present invention is directed to overprodn. of nitric oxide synthase (NOS) in a prokaryote. More particularly, the invention involves overexpression of functional neuronal NOS in a **protease**-deficient strain of Escherichia coli, utilizing a pCW vector under the control of the tac promotor. The invention further involves co-expression of the protein with folding agonists, or chaperonins. The enzyme produced is complete with heme and flavins, and may be activated by incubation with tetrahydrobiopterin. It may be isolated as a predominantly high spin heme protein that demonstrates spectral properties which are identical to those of nNOS isolated from human kidney 293 cells. The methods disclosed are contemplated to be useful in expressing large amts. of other nitric oxide synthases, as well as other proteins that are difficult to produce correctly folded in prokaryotes.

IT **9001-92-7, Proteinase**
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(**protease**-deficient prokaryote host cell; overprodn. of human neuronal nitric oxide synthase in prokaryote using coexpression of folding agonists)

L4 ANSWER 19 OF 25 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1995:945640 HCAPLUS
DOCUMENT NUMBER: 123:332944
TITLE: Topogenesis of cytochrome oxidase subunit II. Mechanisms of protein export from the mitochondrial matrix
AUTHOR(S): Herrmann, Johannes M.; Koll, Hans; Cook, Robert A.; Neupert, Walter; Stuart, Rosemary A.
CORPORATE SOURCE: Inst. Physiologische Chemie, Univ. Muenchen, Munich, 80336, Germany
SOURCE: Journal of Biological Chemistry (1995), 270(45), 27079-86
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Bio logy
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Cytochrome c oxidase subunit II (COXII) in yeast mitochondria is synthesized as a precursor (preCOXII) and is sorted across the inner membrane, whereby both N and C termini become exposed to the intermembrane space. The authors describe here how this process can be exptl. dissected into a no. of distinct stages. The results demonstrate that the translation of COXII is not obligatorily coupled to translocation. Insertion into the inner membrane and export of the N- and C-terminal domains require an energized inner membrane. The export of COXII is independent of both maturation by the Imp1p **protease** and assembly into the cytochrome c oxidase complex. When linked to a mitochondrial matrix-targeting sequence, the N-terminal portion of preCOXII (fused to mouse dihydrofolate reductase) can be imported into the mitochondrial matrix. Following accumulation in the matrix, this chimeric protein can become exported across the inner membrane, delivering the N terminus into the intermembrane space where it undergoes processing by the Imp1p **protease**. This export process displays a no.

of similarities to **bacterial** protein export and supports the view that the principles of sorting are conserved from **prokaryotes** to eukaryotic organelles.

L4 ANSWER 20 OF 25 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1995:193190 HCAPLUS
DOCUMENT NUMBER: 122:206238
TITLE: Absence of Helicobacter pylori in subgingival samples determined by polymerase chain reaction
AUTHOR(S): Asikainen, S.; Chen, C.; Slots, J.
CORPORATE SOURCE: Department Periodontology, University Southern California, Los Angeles, CA, USA
SOURCE: Oral Microbiology and Immunology (1994), 9(5), 318-20
CODEN: OMIMEE; ISSN: 0902-0055
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The polymerase chain reaction was used for the detection of Helicobacter pylori from subgingival plaque in 336 periodontitis patients. A pair of primers derived from the H. pylori urease gene A served to amplify a targeted 411-bp fragment of genomic DNA. This technique permitted the detection of as few as 60 H. pylori cells. Paper point samples from 3 deep periodontal pockets per patient were immersed in 1 mL of phosphate-buffered saline or distd. water, DNA was solubilized by detergent/**protease** method, 3.7 .mu.L or 37 .mu.L of lysate supernatant was used as template, and the amplification product was analyzed in a 1% agarose gel contg. ethidium bromide. Each expt. included purified DNA and cell lysate of H. pylori as pos. controls. The presence of **bacteria** in the sample was verified by a primer pair common to **prokaryote** 16S rRNA. The present study did not reveal the specific polymerase chain reaction amplification product characteristic of H. pylori. It was concluded that periodontal pockets do not constitute a natural reservoir for H. pylori.

L4 ANSWER 21 OF 25 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1991:202360 HCAPLUS
DOCUMENT NUMBER: 114:202360
TITLE: Purification and properties of an intracellular calmodulinlike protein from Bacillus subtilis cells
AUTHOR(S): Fry, Ilona J.; Becker-Hapak, Michelle; Hageman, James H.
CORPORATE SOURCE: Dep. Chem., New Mexico State Univ., Las Cruces, NM, 88003, USA
SOURCE: Journal of Bacteriology (1991), 173(8), 2506-13
CODEN: JOBAAY; ISSN: 0021-9193
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Although calcium ions are crucial in a variety of **bacterial** processes, including spore development, reports of calmodulin in **prokaryotes** have been few. Calmodulinlike protein (CaLP) was purified to homogeneity from sporulating cells of B. subtilis grown in a chem. defined sporulation medium; purifn. involved heat treatment, fractionation with ammonium sulfate, affinity chromatog., and gel filtration on high-performance columns. The protein was eluted from a phenothiazine affinity column in a calcium ion-dependent manner, stained poorly with Coomassie blue and silver

stain dyes, bound poorly to nitrocellulose filters, and was not an inhibitor of the major intracellular serine **proteinase**. It stimulated bovine brain phosphodiesterase in a dose- and Ca2+-dependent manner and stimulated NAD kinase from peas in a dose-dependent manner. The B. subtilis calmodulin reacted with anti-bovine brain calmodulin antibodies in enzyme-linked immunoabsorbance assays. The amino acid compn. data showed it to be distinctly different from eukaryotic calmodulins, having particularly high levels of serine and glycine. The pI of the protein was 4.9-5.0. The mol. wt. was 23,000 to 25,000 based on amino acid compn. and detergent gel electrophoresis, resp. The protein reacted with rhodamine isothiocyanate, which blocked its enzyme-activating capacity and greatly increased its electrophoretic mobility and Coomassie dye-binding ability.

L4 ANSWER 22 OF 25 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1989:434690 HCAPLUS
DOCUMENT NUMBER: 111:34690
TITLE: Signal sequence derived from genes encoding proteses A and B of Streptomyces griseus for recombinant protein scretion
INVENTOR(S): Garvin, Robert T.; Henderson, Graham; Krygsman, Phyllis; Liu, Ci Jun; Davey, Cheryl; Malek, Lawrence T.
PATENT ASSIGNEE(S): Cangene Corp., Can.
SOURCE: Eur. Pat. Appl., 25 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 300466	A1	19890125	EP 1988-111713	19880720
EP 300466	B1	19950913		
R: AT, BE, CH, DE, ES, FR, GB, GR, IT, LI, LU, NL, SE				
CA 1295566	A1	19920211	CA 1987-542678	19870721
ES 2076152	T3	19951101	ES 1988-111713	19880720
US 5514590	A	19960507	US 1994-203644	19940301
US 5641663	A	19970624	US 1994-318193	19941005

PRIORITY APPLN. INFO.:

CA 1987-542678	19870721
US 1985-795331	19851106
US 1988-221346	19880718
CA 1988-572956	19880725
US 1988-224568	19880726
US 1991-646466	19910125
US 1992-844937	19920304
US 1992-863546	19920406
US 1992-935314	19920826
US 1993-66938	19930525

AB DNA sequences of S. griseus encoding 38 amino acid signal peptides are isolated for use in directing the secretion of recombinant proteins, esp., enzymes catalyzing the formation of disulfide bonds such as disulfide oxidoreductase. Genes sprA and sprB of S. griseus ATCC 15395 were cloned and sequenced. The signal sequences of these preproprotease A and preproprotease B genes were identified.

L4 ANSWER 23 OF 25 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1989:109447 HCAPLUS
DOCUMENT NUMBER: 110:109447
TITLE: Stable gene amplification in chromosomal DNA of **prokaryotic microorganisms** and use of the resulting transformants for manufacture of proteins
INVENTOR(S): Van Eekelen, Christiaan Albertus; Van der Laan, Johannes Cornelis; Mulleners, Leonardus Johannes Sofie Marie
PATENT ASSIGNEE(S): Gist-Brocades N. V., Neth.
SOURCE: PCT Int. Appl., 61 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 8806623	A1	19880907	WO 1988-NL6	19880226
W: AU, BG, BR, DK, FI, HU, JP, KR, NO, SU				
AU 8813981	A1	19880926	AU 1988-13981	19880226
AU 620326	B2	19920220		
JP 01502398	T2	19890824	JP 1988-502471	19880226
JP 2637532	B2	19970806		
BR 8805646	A	19891017	BR 1988-5646	19880226
HU 50877	A2	19900328	HU 1988-1833	19880226
RU 2091487	C1	19970927	RU 1988-4356795	19880226
CN 1030787	A	19890201	CN 1988-101680	19880227
EP 284126	A1	19880928	EP 1988-200376	19880229
EP 284126	B1	19930811		
R: AT, BE, CH, DE, ES, FR, GB, GR, IT, LI, LU, NL, SE				
AT 92963	E	19930815	AT 1988-200376	19880229
ES 2045081	T3	19940116	ES 1988-200376	19880229
CA 1327175	A1	19940222	CA 1988-560122	19880229
NO 8804422	A	19881005	NO 1988-4422	19881005
KR 9700188	B1	19970106	KR 1988-71325	19881021
FI 8804903	A	19881024	FI 1988-4903	19881024
DK 8805963	A	19881027	DK 1988-5963	19881027
LV 10791	B	19951220	LV 1993-1313	19931208
LT 4001	B	19960625	LT 1994-1826	19940128
US 5733723	A	19980331	US 1994-295082	19940824
US 6124097	A	20000926	US 1998-49867	19980327

PRIORITY APPLN. INFO.:

EP 1987-200356	A	19870227
WO 1988-NL6	A	19880226
EP 1988-200376	A	19880229
US 1988-162105	B1	19880229
US 1991-653977	B1	19910211
US 1992-893601	B1	19920603
US 1994-295082	A1	19940824

AB Stably transformed **prokaryotes** which can be used to produce increased quantities of a desired protein (relative to the nontransformed **microbe**) are prepd. The transformant contains .gtoreq.2 copies of the gene encoding the desired protein, which copies are sepd. by endogenous chromosomal DNA. The serine **protease** gene of Bacillus novo PB92 was cloned and sequenced. Plasmid pMax-4, contg. this gene as well as the neo gene

and temp.-sensitive replicon of pE194neo, was prepd. Transformation of Bacillus PB92 with this plasmid followed by serial cultivation in media contg. neomycin 20 .mu.g/mL resulted in strain PBT108, in which the serine **protease** gene was stably integrated into the genome as a result of illegitimate recombination. After 2 days culturing, 100% of the population were still neomycin resistant. These transformants produced 120% of the **protease** that the parent strain did.

IT **9001-92-7, Protease 37259-58-8,** Serine **protease**
RL: PRP (Properties)
(gene for, prokaryotes stably transformed with, chromosomal integration of gene in relation to)

L4 ANSWER 24 OF 25 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1982:420458 HCAPLUS
DOCUMENT NUMBER: 97:20458
TITLE: Predominant role of hydrocarbon solubilization in the microbial uptake of hydrocarbons
AUTHOR(S): Reddy, P. G.; Singh, H. D.; Roy, P. K.; Baruah, J. N.
CORPORATE SOURCE: Biochem. Div., Reg. Res. Lab., Assam, 785006, India
SOURCE: Biotechnology and Bioengineering (1982), 24(6), 1241-69
CODEN: BIBIAU; ISSN: 0006-3592
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Using EDTA and proteolytic enzymes to suppress hydrocarbon solubilization, direct evidence is presented in support of the mechanism of liq. hydrocarbon uptake by microbial cells predominantly from the solubilized or accommodated substrate. EDTA (2-5 mM) strongly inhibited growth of 3 yeast species and 1 bacterial species on n-hexadecane and the inhibition was removed by surfactant-emulsified and surfactant-solubilized alkane and also by excess addn. of Ca2+. EDTA had no inhibitory effect on the growth of the organisms on sol. substrates such as NaOAc and nutrient broth or on n-pentane, a volatile alkane which was primarily transported by diffusion from gas phase. EDTA had no significant effect on the adsorption of cells on alkane drops. EDTA inhibition of growth was due to suppression of alkane solubilization, brought about by the solubilizing factor(s) produced by cells. EDTA did not inhibit the growth of yeast on solubilized alkane but strongly inhibited its growth on alkane drops. Adherent capacity of microbial cells to oil phase was closely related to the state of hydrocarbon emulsification and had no relation to the ability of organisms to grow on hydrocarbon. Certain proteolytic enzymes inhibited the growth of yeast on alkane, presumably by digesting the alkane solubilizing protein, but not on glucose, and the inhibition was removed by a supply of surfactant-emulsified and surfactant-solubilized alkane. Specific solubilization of various hydrocarbon types during growth of the **prokaryotic bacterial** strain was demonstrated. The specific solubilization of hydrocarbon was strongly inhibited by EDTA, and the inhibition was removed by excess Ca2+. Apparently, specific solubilization of hydrocarbons is an important mechanism in the microbial uptake of hydrocarbons.

IT **9001-92-7**
RL: BIOL (Biological study)

(hydrocarbon solubilization suppression by, uptake by microorganisms in relation to)

L4 ANSWER 25 OF 25 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1981:134955 HCAPLUS
DOCUMENT NUMBER: 94:134955
TITLE: Crayfish trypsin: missing link between procaryote and mammalian serine **proteases**
AUTHOR(S): Zwilling, R.; Neurath, H.; Woodbury, R. G.
CORPORATE SOURCE: Inst. Zool., Univ. Heidelberg, Heidelberg, Fed. Rep. Ger.
SOURCE: Protides Biol. Fluids (1980), 28th, 115-18
CODEN: PBFPA6; ISSN: 0079-7065
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Crayfish (Astacus fluviatilis) trypsin is an invertebrate serine **protease** that has preserved many characteristics of an early stage of serine **protease** evolution. Since this invertebrate trypsin shares some essential structural properties with **bacterial** trypsin rather than with bovine trypsin, it might be considered a missing link between **prokaryote** and mammalian serine **proteases**.

(FILE 'MEDLINE, BIOSIS, EMBASE, WPIDS, CONFSCI, SCISEARCH, JICST-EPLUS, JAPIO' ENTERED AT 14:15:41 ON 25 NOV 2002)
L5 308 S L4
L6 50 S L5 AND SUBSTRATE
L7 34 DUP REM L6 (16 DUPLICATES REMOVED)

L7 ANSWER 1 OF 34 WPIDS (C) 2002 THOMSON DERWENT
ACCESSION NUMBER: 2002-340184 [37] WPIDS
CROSS REFERENCE: 1999-095351 [08]; 2001-146289 [15]; 2001-367710 [38]; 2002-017124 [02]; 2002-017125 [02]; 2002-017215 [02]; 2002-194904 [25]; 2002-239225 [29]; 2002-697263 [75]
DOC. NO. CPI: C2002-097844
TITLE: Identifying polynucleotide in liquid phase comprises contacting polynucleotides derived from organism with nucleic acid probe labelled with detectable molecule and identifying polynucleotide.
DERWENT CLASS: A89 B04 D15 D16
INVENTOR(S): LAFFERTY, W M; KELLER, M; SHORT, J M
PATENT ASSIGNEE(S): (DIVE-N) DIVERSA CORP; (LAFF-I) LAFFERTY W M
COUNTRY COUNT: 97
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2002031203	A2	20020418	(200237)*	EN	228
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZW					
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PH PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW					
US 2002048809	A1	20020425	(200245)		

AU 2002011642 A 20020422 (200254)

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2002031203 A2		WO 2001-US31806	20011010
US 2002048809 A1	CIP of	US 1997-876276	19970616
	Cont of	US 1998-98206	19980616
	CIP of	US 1999-444112	19991122
	CIP of	US 2000-636778	20000811
	CIP of	US 2000-687219	20001012
		US 2001-790321	20010221
AU 2002011642 A		AU 2002-11642	20011010

FILING DETAILS:

PATENT NO	KIND	PATENT NO
AU 2002011642 A	Based on	WO 200231203

PRIORITY APPLN. INFO: US 2001-309101P 20010731; US 2000-685432 20001010; US 2000-738871 20001215; US 2001-790321 20010221; US 2001-894956 20010627; US 1997-876276 19970616; US 1998-98206 19980616; US 1999-444112 19991122; US 2000-636778 20000811; US 2000-687219 20001012

AN 2002-340184 [37] WPIDS

CR 1999-095351 [08]; 2001-146289 [15]; 2001-367710 [38]; 2002-017124 [02]; 2002-017125 [02]; 2002-017215 [02]; 2002-194904 [25]; 2002-239225 [29]; 2002-697263 [75]

AB WO 200231203 A UPAB: 20021120

NOVELTY - Identifying a polynucleotide in a liquid phase comprises contacting polynucleotides derived from at least one organism with at least one nucleic acid probe labelled with detectable molecule so that the probe is hybridized to the polynucleotides having complementary sequences and identifying a polynucleotide with an analyzer to detect the detectable molecule.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the following:

(1) identifying a polynucleotide encoding a polypeptide which comprises coencapsulating in a microenvironment a library of clones containing DNA obtained from a mixed population of organisms with a mixture of oligonucleotide probes comprising a detectable label and at least a part of a polynucleotide sequence encoding a polypeptide having a specified bioactivity under conditions and for a time to allow interaction of complementary sequences and identifying clones containing a complement to the oligonucleotide probe encoding the polypeptide by separating clones with an analyzer to detect the detectable label;

(2) high throughput screening of a polynucleotide library for a polynucleotide that encodes a molecule which comprises contacting a library containing clones comprising polynucleotides derived from a mixed population of organisms with oligonucleotides probes labelled with a detectable molecule and separating clones with an analyzer to detect the molecule;

(3) screening for a polynucleotide encoding an activity which

comprises:

(a) normalizing polynucleotides obtained from an environmental sample;

(b) generating a library from the polynucleotides;

(c) contacting the library with oligonucleotide probes comprising a detectable label and at least a part of a polynucleotide sequence encoding a polypeptide having a specified activity to select library clones positive for a sequence and

(d) selecting clones with an analyzer to detect the label;

(4) screening polynucleotides which comprises contacting a library of polynucleotides derived from a mixed population of organisms with a probe oligonucleotide labelled with a fluorescence molecule which fluoresces upon binding of the probe to a target polynucleotide of the library to select library polynucleotides positive for a sequence, separating library members that are positive for the sequence with a fluorescent analyzer to detect fluorescence and expressing the selected polynucleotides to obtain polypeptides;

(5) obtaining an organism from a mixed population of organisms in a sample which comprises encapsulating at least one organism from the sample in a microenvironment, incubating under conditions and for a time to allow the organism to grow or proliferate and sorting the organism by a flow cytometer;

(6) identifying a bioactivity or biomolecule which comprises transferring a library containing clones comprising polynucleotides derived from a mixed population of organisms to a first host cell, contacting the cell with a second host cell containing a detectable reporter molecule in a microenvironment and separating clones with an analyzer to detect the molecule;

(7) identifying a bioactivity or biomolecule which comprises transferring a library containing clones comprising polynucleotides derived from a mixed population of organisms to a first host cell, contacting the cell with a second host cell containing a detectable reporter molecule in a microenvironment and optionally separating clones with an analyzer to detect the molecule;

(8) identifying a bioactivity or biomolecule which comprises transferring the extract of a library containing clones comprising polynucleotides derived from a mixed population of organisms to a first host cell and contacting the extract with a second host cell containing a detectable reporter molecule;

(9) identifying a bioactivity or biomolecule which comprises transferring the extract of a library containing clones comprising polynucleotides derived from a mixed population of organisms through a column, transferring the extract to a first host cell, contacting the extract with a second host cell containing a detectable reporter molecule and measuring the mass spectra of the host cell with the extract;

(10) a sample screening apparatus which comprises an array of capillaries comprising at least one wall defining a lumen for retaining a sample, interstitial material between capillaries and at least one reference indicia formed within the interstitial material;

(11) a capillary for screening a sample which comprises a first wall defining a lumen for retaining the sample and forming a waveguide for propagating detectable signals and a second wall formed of a filtering material for filtering excitation energy to the lumen to excite the sample;

(12) a capillary array for screening samples which comprises capillaries as above;

(13) incubating a bioactivity or biomolecule which comprises introducing a first component into at least a part of a capillary of a capillary array, introducing air into the capillary behind the first component and introducing a second component into the capillary;

(14) incubating a sample which comprises introducing a first liquid labelled with a detectable particle into a capillary of a capillary array, optionally with at least one wall coated with a binding material, submersing one end of the capillary into a fluid bath containing a second liquid and evaporating the first liquid;

(15) incubating a sample which comprises introducing a liquid labelled with a detectable particle into a capillary of a capillary array, introducing paramagnetic beads to the liquid and exposing the capillary containing the beads to a magnetic field;

(16) recovering a sample from one capillary in an array which comprises determining a coordinate position of a recovery tool, detecting a coordinate location of a capillary containing the sample, correlating, via relative movement between the recovery tool and the capillary containing the sample, the coordinate position of the recovery tool with the location of the capillary and contacting the capillary and recovery tool;

(17) a recovery apparatus which comprises a recovery tool to contact at least one capillary and recover a sample and an ejector, connected with the recovery tool, for ejecting the sample from the tool;

(18) a sample screening apparatus which comprises capillaries in an array, interstitial material and at least one reference indicia formed within the interstitial material, and

(19) enriching a polynucleotide encoding an activity which comprises contacting a mixed population of polynucleotides derived from a mixed population of organisms with at least one nucleic acid probe.

USE - Used for screening for polynucleotides, proteins and small molecules using high throughput of multiple samples.

ADVANTAGE - Rapid sorting and screening of libraries from a mixed population of organisms may be effected.

Dwg.0/23

L7 ANSWER 2 OF 34 WPIDS (C) 2002 THOMSON DERWENT

ACCESSION NUMBER: 2002-393965 [42] WPIDS

CROSS REFERENCE: 1997-145245 [13]; 1997-319766 [29]; 1998-101069 [09]; 1998-609243 [51]; 1999-263358 [22]; 1999-313351 [26]; 2000-524416 [47]; 2000-587434 [55]; 2000-594650 [56]; 2001-050094 [06]; 2002-083006 [11]

DOC. NO. CPI: C2002-110849

TITLE: Obtaining bioactivity/biomolecule of interest by screening library of clones generated from nucleic acids from mixed cell population, and variegating nucleic acids to create novel biomolecule/bioactivity of interest.

DERWENT CLASS: B04 D16

INVENTOR(S): SHORT, J M

PATENT ASSIGNEE(S): (DIVE-N) DIVERSA CORP

COUNTRY COUNT: 97

PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG

WO 2002022810 A2 20020321 (200242)* EN 154
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC
MW MZ NL OA PT SD SE SL SZ TR TZ UG ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ
DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP
KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ
NO NZ PH PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG
US UZ VN YU ZA ZW
AU 2001091208 A 20020326 (200251)

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2002022810 A2		WO 2001-US29712	20010917
AU 2001091208 A		AU 2001-91208	20010917

FILING DETAILS:

PATENT NO	KIND	PATENT NO
AU 2001091208 A	Based on	WO 200222810

PRIORITY APPLN. INFO: US 2000-663620 20000915
AN 2002-393965 [42] WPIDS
CR 1997-145245 [13]; 1997-319766 [29]; 1998-101069 [09]; 1998-609243 [51]; 1999-263358 [22]; 1999-313351 [26]; 2000-524416 [47]; 2000-587434 [55]; 2000-594650 [56]; 2001-050094 [06]; 2002-083006 [11]
AB WO 200222810 A UPAB: 20021120
NOVELTY - Obtaining (M1) a bioactivity or biomolecule (BB) of interest, comprising:
(a) screening a library of clones generated from nucleic acids (NA) from a mixed population of cells, for a specified BB;
(b) variegating NA contained in clone with a specified BB; and
(c) comparing BB from (b) with the specified BB where a difference in BB is indicative of an effect of sequence variegation, is new.
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:
(1) identifying bioactivity or a biomolecule of interest, comprises (M2-M4):
(a) screening a library of clones generated from pooled NA obtained from several isolates for a specified BB and identifying a clone which contains the specified BB (M1);
(b) screening a library of clones generated from pooling individual gene libraries generated from the nucleic acids obtained from each of several of isolates for a specified BB and identifying a clone which contains the specified BB (M2); or
(c) screening a library of clones generated from the nucleic acids from an enriched population of organisms for a specified BB and identifying a clone containing the specified BB (M4), where (M2-4) optionally involve the following steps after the primary screening step:
(i) variegating a nucleic acid sequence contained in a clone having the specified BB; and
(ii) comparing the BB from the above step with the specified

BB, where the difference in the BB is indicative of an effect of introducing at least one sequence variegation, thereby providing the BB of interest;

(2) identifying (M5) a BB of interest comprises:

(a) incubating nucleic acids from a mixed population of organisms with at least one oligonucleotide probe (P) comprising a detectable molecule and at least a portion of a nucleic acid sequence encoding a molecule of interest under such conditions and such time to allow interaction of complementary sequences;

(b) identifying nucleic acid sequences having a complement to the oligonucleotide probe using an analyzer that detects the detectable molecule;

(c) generating a library from the identified nucleic acid sequences;

(d) screening the library for a specified BB;

(e) variegating a nucleic acid sequence contained in a clone having the specified BB; and

(f) comparing the BB product from the above step with the specified BB, where a difference in the BB is indicative of an effect of introducing at least one sequence variation, thereby providing the BB of interest;

(3) identifying (M6) a BB of interest, involves co-encapsulating in a microenvironment nucleic acids obtained from a mixed population of organisms, with (P) to allow interaction of complementary sequences; identifying encapsulated nucleic acids containing complement to (P) encoding the molecule of interest by separating the encapsulated nucleic acids with an analyzer that detects the detectable molecule; and then carrying out steps (c)-(f) as described above;

(4) identifying (M7) a BB of interest involves co-encapsulating in a microenvironment nucleic acids obtained from an isolate of mixed population of organisms, with (P) to allow interaction of complementary sequences; and carrying out steps (b)-(f) as described above;

(5) obtaining (M8) a BB of interest involves co-encapsulating in a microenvironment nucleic acids obtained from one or more isolates of a mixed population of organisms, with (P) to allow interaction of complementary sequences; and carrying out steps (b)-(f) as described above; and

(6) identifying (M9) a BB of interest involves co-encapsulating in a microenvironment nucleic acids obtained from a mixture of isolates of a mixed population of organisms, with (P) to allow interaction of complementary sequences; and carrying out steps (b)-(f) as described above.

USE - The methods are useful for obtaining bioactivity or a biomolecule of interest, preferably a nucleic acid sequence. The method is also useful for obtaining bioactivity provided by a polypeptide e.g. enzymatic activity provided by an enzyme such as lipases, esterases, **proteases**, glycosidases, glycosyl transferases, phosphatases, kinases, mono- and dioxygenases, haloperoxidases, lignin peroxidases, diarylpropane peroxidases, epozide hydrolases, nitrile hydratases, nitrilases, transaminases, amidases, or acylases. The method is also useful for obtaining a BB which is a gene cluster or its fragment, or is a polypeptide in a metabolic pathway (all claimed).

ADVANTAGE - The method combines direct cloning of genes encoding novel or desired bioactivities from environmental samples with a high throughput screening system designed for rapid activity

of new molecules. The method allows rapid screening of complex environmental libraries containing genomic sequences from thousands of genomic organisms or subsets and isolates. The method represents an extremely high throughput screening method which allows one to assess this enormous number of clones. The method allows the screening of 30-200 million clones/hour for a desired nucleic acid sequence, biological activity or biomolecule of interest which allows through screening of environmental libraries for clones expressing novel bioactivities or biomolecules. The method combines the benefits associated with the ability to rapidly screen natural compounds with the flexibility and reproducibility afforded by working with the genetic material of organisms.

DESCRIPTION OF DRAWING(S) - The figure shows site-saturation mutagenesis.

Dwg.1/17

L7 ANSWER 3 OF 34 WPIDS (C) 2002 THOMSON DERWENT
ACCESSION NUMBER: 2002-303940 [34] WPIDS
DOC. NO. CPI: C2002-088355
TITLE: Detection of bacterial contamination in foods or food-related work areas comprises identifying a protein specific to the microorganism using fluorescence or colorimetric methods.
DERWENT CLASS: A89 D13 D16 D22
INVENTOR(S): SANDERS, M C
PATENT ASSIGNEE(S): (EXPR-N) EXPRESSIVE CONSTRUCTS INC
COUNTRY COUNT: 94
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2002010433	A2	20020207	(200234)*	EN	25
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZW					
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG UZ VN YU ZA ZW					
AU 2001096211	A	20020213	(200238)		

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2002010433	A2	WO 2001-US14613	20010503
AU 2001096211	A	AU 2001-96211	20010504

FILING DETAILS:

PATENT NO	KIND	PATENT NO
AU 2001096211	A Based on	WO 200210433

PRIORITY APPLN. INFO: US 2000-201405P 20000503
AN 2002-303940 [34] WPIDS
AB WO 200210433 A UPAB: 20020528
NOVELTY - A specific and sensitive detection of the presence or

absence of pathogenic microorgansism in potentially contaminated food products at the retail level, by detecting a bacteria-specific protein

DETAILED DESCRIPTION - The presence or absence of a **prokaryotic microorganism** is detected in a sample, by (a) identifying a **protease** unique to the **prokaryotic microorganism**, (b) providing a quenched labelled **substrate** specific for this, (c) providing the sample, and (d) determining the presence or absence of a label.

INDEPENDENT CLAIMS are also included for:

(1) similarly detecting pathogenic microorganisms in a sample;

(2) a method of using a broad spectrum fluoresecent or colorimetric labeled peptides to recognize a bacterial species by detecting conjugated peptide with a colorimeter or fluorimeter;

(3) a device for capturing and releasing bacteria from solid or liquid extracts comprising protein encapsulated starch or Styrofoam (RTM);

(4) a sensor for detecting bacteria in a sample, comprising packaging material having a side proximal to the sample, a second side, and a dye labeled **substrate** for the bacteria attached to the first side; and

(5) a method for using alpha-crystalline type protein, by expressing and purifying recombinant alpha-crystalline, and adding this to a solid or liquid phase assay containing a dye labeled peptide in amount to reduce its proteolysis.

USE - The process is useful for detecting Listeria monocytogenes or other food contaminants in food products or food-related work areas

ADVANTAGE - The process is specific and sensitive, yielding a visible color change

Dwg.0/3

L7 ANSWER 4 OF 34 WPIDS (C) 2002 THOMSON DERWENT

ACCESSION NUMBER: 2002-194904 [25] WPIDS

CROSS REFERENCE: 1999-095351 [08]; 2001-146289 [15]; 2001-367710 [38]; 2002-017124 [02]; 2002-017125 [02]; 2002-017215 [02]; 2002-340184 [37]

DOC. NO. NON-CPI: N2002-148026

DOC. NO. CPI: C2002-060165

TITLE: Identifying bioactivities or biomolecules by screening clones from a gene library generated from more than one organism.

DERWENT CLASS: B04 C07 D16 S03

INVENTOR(S): KELLER, M; SHORT, J M

PATENT ASSIGNEE(S): (DIVE-N) DIVERSA CORP

COUNTRY COUNT: 1

PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
US 2002001809	A1	20020103	(200225)*		40

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
US 2002001809	A1 CIP of	US 1997-876276	19970616

Cont of	US 1998-98206	19980616
Div ex	US 2000-636778	20000811
	US 2001-848095	20010503

FILING DETAILS:

PATENT NO	KIND	PATENT NO
US 2002001809 A1	Cont of	US 6174673

PRIORITY APPLN. INFO: US 1998-98206 19980616; US 1997-876276 19970616; US 2000-636778 20000811; US 2001-848095 20010503

AN 2002-194904 [25] WPIDS

CR 1999-095351 [08]; 2001-146289 [15]; 2001-367710 [38]; 2002-017124 [02]; 2002-017125 [02]; 2002-017215 [02]; 2002-340184 [37]

AB US2002001809 A UPAB: 20020717

NOVELTY - A method for identifying bioactivities or biomolecules, comprising inserting a bioactive **substrate** into clones from a gene library generated from more than one organism and screening the clones for a change in the **substrate**, is new.

DETAILED DESCRIPTION - A method for identifying bioactivities or biomolecules using high-throughput screening of nucleic acids comprising:

(a) providing a gene library comprising several clones (the nucleic acid for generating the library is obtained from more than one organism);

(b) inserting a bioactive **substrate** into the clones (a bioactivity or biomolecule produced by the clones is detectable by a difference in the **substrate** before and after contact with the clones);

(c) screening the clones with an assay or analyzer that detects a bioactivity or biomolecule; and

(d) identifying clones detected as positive for a change in the **substrate** (a change in the **substrate** is indicative of DNA that encodes a bioactivity or biomolecule).

USE - The method is especially useful for identifying enzymes in extremophiles, especially where the enzymes are lipases, esterases, **proteases**, glycosidases, glycosyl transferases, phosphatases, kinases, mono- and dioxygenases, haloperoxidases, lignin peroxidases, diarylpropane peroxidases, epoxide hydrolases, nitrile hydratases, nitrilases, transaminases, amidases or acylases, and the extremophiles are thermophiles, hyperthermophiles, psychrophiles, halophiles, psychrotrophs, alkalophiles or acidophiles.

ADVANTAGE - The method can be applied to nucleic acids isolated directly or indirectly from the environment using flow cytometry systems normally used for sorting eukaryotic cells.

Dwg.0/18

L7 ANSWER 5 OF 34 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.

ACCESSION NUMBER: 2002:486567 BIOSIS

DOCUMENT NUMBER: PREV200200486567

TITLE: Molecular evolution of proteasomes.

AUTHOR(S): Volker, C. (1); Lupas, A. N.

CORPORATE SOURCE: (1) Bioinformatics, SmithKline Beecham Pharmaceuticals, 1250 South Collegeville Road, UP

1345, Collegeville, PA, 19426-0989 USA
SOURCE: Zwickl, Peter; Baumeister, Wolfgang. Current Topics in Microbiology and Immunology, (2002) Vol. 268, pp. 1-22. Current Topics in Microbiology and Immunology. The proteasome-ubiquitin protein degradation pathway. print.
Publisher: Springer-Verlag New York Inc. 175 Fifth Avenue, New York, NY, 10010-7858, USA.
ISSN: 0070-217X. ISBN: 3-540-43096-2 (cloth).
DOCUMENT TYPE: Book
LANGUAGE: English

L7 ANSWER 6 OF 34 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
DUPLICATE 1
ACCESSION NUMBER: 2002:535887 BIOSIS
DOCUMENT NUMBER: PREV200200535887
TITLE: Conservation of intramembrane proteolytic activity and **substrate** specificity in prokaryotic and eukaryotic Rhomboids.
AUTHOR(S): Urban, Sinisa; Schlieper, Daniel; Freeman, Matthew (1)
CORPORATE SOURCE: (1) MRC Laboratory of Molecular Biology, Hills Road, Cambridge, CB2 2QH: mf1@mrc-lmb.cam.ac.uk UK
SOURCE: Current Biology, (September 3, 2002) Vol. 12, No. 17, pp. 1507-1512. http://www.current-biology.com/. print.
ISSN: 0960-9822.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Rhomboid is an intramembrane serine **protease** responsible for the proteolytic activation of Drosophila epidermal growth factor receptor (EGFR) ligands (1). Although nothing is known about the function of the apprx100 currently known rhomboid genes conserved throughout evolution, a recent analysis suggests that a Rhomboid from the pathogenic **bacterium** Providencia stuartii is involved in the production of a quorum-sensing factor (2). This suggests that an intercellular signaling mechanism may have been conserved between **prokaryotes** and metazoans (3). However, the function of **prokaryotic** Rhomboids is unknown. We have examined the ability of eight **prokaryotic** Rhomboids to cleave the three Drosophila EGFR ligands. Despite their striking sequence divergence, Rhomboids from one Gram-positive and four Gram-negative species, including Providencia, specifically cleaved Drosophila **substrates**, but not similar proteins such as Transforming Growth Factor alpha (TGFalpha) and Delta. Although the sequence similarity between these divergent Rhomboids is very limited, all contain the putative serine catalytic triad residues, and their specific mutation abolished **protease** activity. Therefore, despite low overall homology, the Rhomboids are a family of ancient, functionally conserved intramembrane serine **proteases**, some of which also have conserved **substrate** specificity. Moreover, a function for Rhomboids in activating intercellular signaling appears to have evolved early.

L7 ANSWER 7 OF 34 MEDLINE
ACCESSION NUMBER: 2002466213 IN-PROCESS
DOCUMENT NUMBER: 22213262 PubMed ID: 12225666
TITLE: Conservation of intramembrane proteolytic activity

and **substrate** specificity in prokaryotic and eukaryotic rhomboids.

AUTHOR: Urban Sinisa; Schlieper Daniel; Freeman Matthew

CORPORATE SOURCE: MRC Laboratory of Molecular Biology, Hills Road, CB2 2QH, Cambridge, United Kingdom.

SOURCE: CURRENT BIOLOGY, (2002 Sep 3) 12 (17) 1507. Journal code: 9107782. ISSN: 0960-9822.

PUB. COUNTRY: England: United Kingdom

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: IN-PROCESS; NONINDEXED; Priority Journals

ENTRY DATE: Entered STN: 20020913
Last Updated on STN: 20020913

AB Rhomboid is an intramembrane serine **protease** responsible for the proteolytic activation of Drosophila epidermal growth factor receptor (EGFR) ligands. Although nothing is known about the function of the approximately 100 currently known rhomboid genes conserved throughout evolution, a recent analysis suggests that a Rhomboid from the pathogenic **bacterium** Providencia stuartii is involved in the production of a quorum-sensing factor. This suggests that an intercellular signaling mechanism may have been conserved between **prokaryotes** and metazoans. However, the function of **prokaryotic** Rhomboids is unknown. We have examined the ability of eight **prokaryotic** Rhomboids to cleave the three Drosophila EGFR ligands. Despite their striking sequence divergence, Rhomboids from one Gram-positive and four Gram-negative species, including Providencia, specifically cleaved Drosophila **substrates**, but not similar proteins such as Transforming Growth Factor alpha (TGFalpha) and Delta. Although the sequence similarity between these divergent Rhomboids is very limited, all contain the putative serine catalytic triad residues, and their specific mutation abolished **protease** activity. Therefore, despite low overall homology, the Rhomboids are a family of ancient, functionally conserved intramembrane serine **proteases**, some of which also have conserved **substrate** specificity. Moreover, a function for Rhomboids in activating intercellular signaling appears to have evolved early.

L7 ANSWER 8 OF 34 SCISEARCH COPYRIGHT 2002 ISI (R)

ACCESSION NUMBER: 2002:687932 SCISEARCH

THE GENUINE ARTICLE: 584GR

TITLE: The crystal structures of four peptide deformylases bound to the antibiotic actinonin reveal two distinct types: A platform for the structure-based design of antibacterial agents

AUTHOR: Guilloteau J P; Mathieu M; Giglione C; Blanc V; Dupuy A; Chevrier M; Gil P; Famechon A; Meinnel T; Mikol V (Reprint)

CORPORATE SOURCE: Drug Innovat & Approvals Aventis Pharma, 13 Quai Jules Guesde, BP 14, F-94403 Vitry Sur Seine, France (Reprint); Drug Innovat & Approvals Aventis Pharma, F-94403 Vitry Sur Seine, France; CNRS, UPR 2355, Inst Sci Vegetal, F-91198 Gif Sur Yvette, France

COUNTRY OF AUTHOR: France

SOURCE: JOURNAL OF MOLECULAR BIOLOGY, (26 JUL 2002) Vol. 320, No. 5, pp. 951-962.
Publisher: ACADEMIC PRESS LTD ELSEVIER SCIENCE LTD, 24-28 OVAL RD, LONDON NW1 7DX, ENGLAND.

ISSN: 0022-2836.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 52

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB **Bacterial** peptide deformylase (PDF) belongs to a sub-family of metallo-**proteases** that catalyse the removal of the N-terminal formyl group from newly synthesised proteins. PDF is essential in **prokaryotes** and conserved throughout the eubacteria. It is therefore considered an attractive target for developing new antibacterial agents. Here, we report the crystal structures of four **bacterial** deformylases, free or bound to the naturally occurring antibiotic actinonin, including two from the major **bacterial pathogens** Pseudomonas aeruginosa and Staphylococcus aureus. The overall tertiary structure is essentially conserved but shows significant differences, namely at the C terminus, which are directly related to the deformylase type (i.e. I or II) they belong to. The geometry around the catalytic metal ion exhibits a high level of similarity within the different enzymes, as does the binding mode of actinonin to the various deformylases. However, some significant structural differences are found in the vicinity of the active site, highlighting the structural and molecular requirements for the design of a deformylase inhibitor active against a broad spectrum of **bacterial** strains. (C) 2002 Elsevier Science Ltd. All rights reserved.

L7 ANSWER 9 OF 34 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.

ACCESSION NUMBER: 2002205266 EMBASE
TITLE: M13 endopeptidases: New conserved motifs correlated with structure, and simultaneous phylogenetic occurrence of PHEX and the bony fish.
AUTHOR: Bianchetti L.; Oudet C.; Poch O.
CORPORATE SOURCE: L. Bianchetti, Inst. Genet./Biol. Molec./Cell., IGBMC, B.P. 163, 67404 Illkirch, France. Laurent.Bianchetti@igbmc.u-strasbg.fr
SOURCE: Proteins: Structure, Function and Genetics, (1 Jun 2002) 47/4 (481-488).
Refs: 56
ISSN: 0887-3585 CODEN: PSFGEY
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English

AB M13 endopeptidase alignments have focused mainly on mammalian sequences and on the active site region defining the catalytic sequence signatures. Aligning all available M13 from **bacteria** to human on a full-length basis, we have performed a sequence analysis. This enabled us to highlight the origin and function of the M13 PHEX subtype family endopeptidase (phosphate regulating gene with homologies to endopeptidases on the X chromosome). New evolutionary conserved regions in both **prokaryotes** and eukaryotes have been detected and eukaryotic-specific regions clearly delineated. Using the recently solved neprilysin structure, we have observed that all new motifs, except one, localize in the spatial vicinity of the previously reported catalytic signatures. Interestingly, a highly hydrophobic

pocket containing three newly reported motifs is centered by the C-terminal tryptophan residue. Extensive M13 searches in complete and in progress higher eukaryotic genomes have lead to the identification of Danio rerio as the simplest organism having PHEX. Finally, the human PHEX **substrate**, the parathyroid hormone-related peptide, PTHrP(107-139), is absent in bony fish: this suggests the existence of further PHEX **substrates** common to both bony fishes and higher vertebrates. .COPYRGT. 2002 Wiley-Liss, Inc.

L7 ANSWER 10 OF 34 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2002:597171 BIOSIS
DOCUMENT NUMBER: PREV200200597171
TITLE: A potential virulence factor chaperone gene of Mycoplasma mycoides subspecies mycoides large colony type.
AUTHOR(S): Rosentel, J. K. (1); Brown, M. B. (1)
CORPORATE SOURCE: (1) University of Florida, Gainesville, FL USA
SOURCE: Abstracts of the General Meeting of the American Society for Microbiology, (2002) Vol. 102, pp. 222. http://www.asmusa.org/mtgsrc/generalmeeting.htm. print.
Meeting Info.: 102nd General Meeting of the American Society for Microbiology Salt Lake City, UT, USA May 19-23, 2002 American Society for Microbiology . ISSN: 1060-2011.
DOCUMENT TYPE: Conference
LANGUAGE: English
AB Mycoplasmas are **prokaryotes** that lack a cell wall and infect a variety of hosts, including humans, mammals, reptiles, fish, arthropods, and plants. Unlike most mycoplasmal infections which cause clinically silent, chronic disease, infections caused by the Mycoplasma mycoides cluster have an acute progression of pneumonia, polyarthritis, mastitis and septicemia that frequently results in disseminated intravascular coagulopathy and death. Five of six organisms that comprise the mycoides cluster produce an extracellular caseinolytic **protease**. The extracellular role of this caseinolytic **protease** is important in **substrate** utilization and microenvironment protection. The intracellular role of this caseinolytic **protease** is still unknown. We investigated this caseinolytic **protease** in Mycoplasma mycoides subspecies mycoides LC type (Mmm) GM12 as a potential virulence factor. A Tn916 insertion mutant, Mmm GM12-8 was selected for tetracycline resistance and the inability to degrade casein. The genomic DNA was isolated, and the flanking regions of the Tn916 insertion were sequenced. This lead to the cloning of the caseinolytic **protease** gene and complete sequence. The putative gene sequence BLAST result revealed homology to a caseinolytic **protease** C (ClpC) ATPase gene of many **microorganisms**. The ClpC ATPase belongs to the heat shock protein family that plays a major chaperone role in the virulence of other **pathogens**, including Listeria **monocytogenes** , Plasmodium malariae, Bacillus anthracis, Ureaplasma urealyticum, and Streptococcus pneumoniae. This data supports the role of the Mmm caseinolytic **protease** as a potential virulence factor chaperone.

L7 ANSWER 11 OF 34 WPIDS (C) 2002 THOMSON DERWENT

ACCESSION NUMBER: 2001-488877 [53] WPIDS
CROSS REFERENCE: 2001-336001 [35]; 2002-415722 [25]
DOC. NO. CPI: C2001-146830
TITLE: Novel single chain polypeptide comprising **protease** domain of type-II membrane-type serine **protease** or its catalytically active portion useful for treating and preventing cancer and tumor.
DERWENT CLASS: B04 D16
INVENTOR(S): MADISON, E L; ONG, E O; YEH, J
PATENT ASSIGNEE(S): (CORV-N) CORVAS INT INC
COUNTRY COUNT: 93
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2001057194	A2	20010809	(200153)*	EN	256

RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZW
W: AE AG AM AT AU AZ BA BB BG BR BY BZ CA CH CN CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW

AU 2001033262	A	20010814	(200173)

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2001057194	A2	WO 2001-US3471	20010202
AU 2001033262	A	AU 2001-33262	20010202

FILING DETAILS:

PATENT NO	KIND	PATENT NO
AU 2001033262	A Based on	WO 200157194

PRIORITY APPLN. INFO: US 2000-234840P 20000922; US 2000-179982P 20000203; US 2000-183542P 20000218; US 2000-213124P 20000622; US 2000-220970P 20000726; US 2000-657986 20000908

AN 2001-488877 [53] WPIDS
CR 2001-336001 [35]; 2002-415722 [25]
AB WO 200157194 A UPAB: 20020711
NOVELTY - A substantially purified single chain polypeptide (I) comprising the **protease** domain of a type-II membrane-type serine **protease** (MTSP) or its catalytically active portion, where the MTSP portion of the protein consists essentially of the **protease** domain of the MTSP or its catalytically active portion, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) a nucleic acid molecule (II) comprising a sequence of nucleotides that encode (I);

(2) a mutein (III) of (I) where up to about 90% of the amino acids, and the resulting polypeptide is a single chain and has

catalytic activity at least 10% of the unmutated polypeptide;

(3) a vector (IV) comprising (II);

(4) a cell (V) comprising (IV);

(5) production of (I);

(6) an antisense nucleic acid molecule (VI) that comprises at least 14, preferably 16 contiguous nucleotides or modified nucleotides that are complementary to a contiguous sequence of nucleotides in the **protease** domain of (I);

(7) an antibody (VII) that specifically binds to the single chain form of a **protease** domain of (I), or its fragment or derivative containing a binding domain, where (VII) is a polyclonal or monoclonal antibody;

(8) a conjugate (VIII) comprising (I), and a targeting agent linked to (I) directly or through a linker;

(9) a combination (IX) comprising an inhibitor of the catalytic activity of (I), and another treatment or agent selected from anti-tumor and anti-angiogenic treatments or agents;

(10) a solid support (X) comprising two or more of (I) linked to it either directly or through a linker;

(11) identifying (M1) compounds that modulate the **protease** activity of an MTSP involves contacting (I) with a **substrate** proteolytically cleaved by the MTSP, and, either simultaneously, before or after, adding a test compound or a number of test compounds, measuring the amount of **substrate** cleaved in the presence of the test compound, and selecting compounds that change the amount cleaved compared to a control, where compounds that modulate the activity of MTSP are identified;

(12) a modulator (XI) of the activity of MTSP identified by M1;

(13) identifying (M2) a compound that specifically binds to a single chain **protease** domain of an MTSP involves contacting (I) with a test compound or number of test compounds under conditions conducive to binding, and identifying compounds that specifically bind to the MTSP single chain **protease** domain, where the known compound is contacted with the polypeptide before, simultaneously with or after the test compound;

(14) a recombinant non-human animal (XII), where an endogenous gene of an MTSP has been deleted or inactivated by homologous recombination or insertional mutagenesis of the animal or its ancestor;

(15) detecting (M3) neoplastic disease involves detecting (I) such as MTSP3, MTSP4, MTSP6 in a biological sample, where the amount detected differs from the amount in a subject who does not have the neoplastic disease; and

(16) treating (M4) tumors involves administering a prodrug that is specifically cleaved by (I), where upon contact with the cell that expresses MTSP activity, the prodrug is converted into an active drug.

ACTIVITY - Cytostatic.

MECHANISM OF ACTION - Modulator of (I) (claimed); gene therapy. No supporting data is given.

USE - (I) is useful for identifying compounds that modulate the activity of (I), where the compounds inhibit proteolytic activity of (I), and for formulating a medicament for treating neoplastic disease (claimed). (I) or (II) is useful in preventing or treating tumors or cancers such as lung carcinoma, colon adenocarcinoma and ovarian carcinoma, in diagnostics, and in hybridization assays . (I) is useful as a diagnostic marker for tumor development, growth, and/or progression, to identify compounds that modulate the activity

of (I), and as immunogens to generate antibodies that specifically bind to it. (II) is useful in a yeast two-hybrid system and in gene therapy. (XII) is useful in animal models of tumor initiation, growth and/or progression models.
Dwg.0/3

L7 ANSWER 12 OF 34 WPIDS (C) 2002 THOMSON DERWENT
ACCESSION NUMBER: 2001-367710 [38] WPIDS
CROSS REFERENCE: 1999-095351 [08]; 2001-146289 [15]; 2002-017124 [02]; 2002-017125 [02]; 2002-017215 [02]; 2002-194904 [25]; 2002-239225 [29]; 2002-340184 [37]; 2002-697263 [75]
DOC. NO. CPI: C2001-112873
TITLE: Identifying a bioactivity or biomolecule of interest, involves culturing a **substrate** labeled with a detectable molecule and a recombinant clone in a capillary tube of capillary array, and detecting the signal.
DERWENT CLASS: B04 D16
INVENTOR(S): KELLER, M; LAFFERTY, W M; SHORT, J M
PATENT ASSIGNEE(S): (DIVE-N) DIVERSA CORP
COUNTRY COUNT: 94
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2001038583	A2	20010531	(200138)*	EN	133

RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG UZ VN YU ZA ZW

AU 2001017961	A	20010604	(200153)		
EP 1144679	A2	20011017	(200169)	EN	

R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SI

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2001038583	A2	WO 2000-US32208	20001122
AU 2001017961	A	AU 2001-17961	20001122
EP 1144679	A2	EP 2000-980740	20001122
		WO 2000-US32208	20001122

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 2001017961	A	Based on	WO 200138583
EP 1144679	A2	Based on	WO 200138583

PRIORITY APPLN. INFO: US 2000-687219 20001012; US 1999-444112 19991122
AN 2001-367710 [38] WPIDS

CR 1999-095351 [08]; 2001-146289 [15]; 2002-017124 [02]; 2002-017125 [02]; 2002-017215 [02]; 2002-194904 [25]; 2002-239225 [29]; 2002-340184 [37]; 2002-697263 [75]

AB WO 200138583 A UPAB: 20021120

NOVELTY - Identifying (I) bioactivity or biomolecule of interest, is new, comprising introducing a **substrate** (S) labeled with a detectable molecule and a recombinant clone (RC) into a capillary tube (T), where each (T) of an array (A) comprises at least one wall defining a lumen for retaining (S) and RC, and culturing (T) containing (S) and (RC), to produce a detectable signal, is new.

DETAILED DESCRIPTION - Identifying (I) bioactivity or biomolecule of interest, is new, comprising introducing a **substrate** (S) labeled with a detectable molecule and a recombinant clone (RC) into a capillary tube (T), where each (T) of an array (A) comprises at least one wall defining a lumen for retaining (S) and RC, and culturing (T) containing (S) and (RC), to produce a detectable signal, is new. The signal in (T) is detected, to identify one or more capillaries containing the detectable signal, and thus identifying bioactivity or biomolecule of interest.

Alternatively, (I) comprises:

(a) introducing RC into (T), where each capillary tube of (A) comprises at least one wall defining a lumen for retaining RC, exposing RC to conditions which induce detectable signal, and detecting the detectable signal in (T); or

(b) introducing RC containing a **substrate** into (T), where each capillary tube of (A) comprises at least one wall defining a lumen for retaining RC containing **substrate**, exposing RC to conditions which cause the **substrate** to produce a detectable signal, and detecting the signal in (T).

INDEPENDENT CLAIMS are also included for the following:

(1) an automated capillary array system comprising:

(a) a number of capillary tubes defining (A), each separated from the other by at least one material having a low refractive index, and each having openings at each end;

(b) a mixer for mixing the contents of (T), or at least one magnetic field apparatus in magnetic communication with (A) to cause movements of paramagnetic beads;

(c) an optical array in optical communication with at least one end of (A) that detects an optical signal produced from a sample in at least one (T); and

(d) a computer system in communication with the mixer or magnetic field apparatus and the optical array, that controls the mixing of capillary array or magnetic field surrounding (A) and processes data detected by the optical array; and

(2) identifying a compound of interest, comprising:

(a) introducing a number of compounds into (T);

(b) exposing the sample to conditions which cause the compound of interest to produce a detectable signal; and

(c) detecting the detectable signal in (T) to identify one or more capillaries containing the detectable signal, thereby identifying the compound of interest.

USE - The method is useful for identifying a bioactivity or biomolecule of interest, where the bioactivity of interest is an activity of enzymes such as lipases, esterases, **proteases**, peptidases, reductases, oxidoreductases, lyases, ligases, isomerases, polymerases, synthases, synthetases, glycosidases, transferases, phosphatases, kinases, mono-and dioxygenases, peroxidases, hydrolases, hydratases, nitrilases, transaminases,

amidases or acylases (claimed).

ADVANTAGE - The method is rapid and efficient. The method combines the benefits associated with the ability to rapidly screen natural compounds with flexibility and reproducibility afforded with working with the genetic material of organism. The method increases the repertoire of available sequences that can be used for the development of diagnostics, therapeutics or molecules for industrial applications. No prior information regarding an expected ligand structure is required to isolate peptide ligands or antibodies. Dwg.0/15

L7 ANSWER 13 OF 34 WPIDS (C) 2002 THOMSON DERWENT
ACCESSION NUMBER: 2001-336001 [35] WPIDS
CROSS REFERENCE: 2001-488877 [53]; 2002-415722 [44]
DOC. NO. CPI: C2001-103895
TITLE: New nucleic acid encoding a protein comprising endotheliase activity useful in the prevention and treatment of e.g. vascular malformations, cardiovascular disorders, and chronic inflammatory disease.
DERWENT CLASS: B04 D16
INVENTOR(S): MADISON, E L; ONG, E O
PATENT ASSIGNEE(S): (CORV-N) CORVAS INT INC
COUNTRY COUNT: 94
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2001036604	A2	20010525	(200135)*	EN	152
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZW					
W: AE AG AM AT AU AZ BA BB BG BR BY BZ CA CH CN CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW					
AU 2001016239	A	20010530	(200152)		
EP 1230349	A2	20020814	(200261)	EN	
R: AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI TR					

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2001036604	A2	WO 2000-US31803	20001117
AU 2001016239	A	AU 2001-16239	20001117
EP 1230349	A2	EP 2000-978819	20001117
		WO 2000-US31803	20001117

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 2001016239	A	Based on	WO 200136604
EP 1230349	A2	Based on	WO 200136604

PRIORITY APPLN. INFO: US 2000-234840P 20000922; US 1999-166391P

19991118
AN 2001-336001 [35] WPIDS
CR 2001-488877 [53]; 2002-415722 [44]
AB WO 200136604 A UPAB: 20020924
NOVELTY - A nucleic acid (N1) comprising a sequence encoding a substantially purified protein (P1) comprising the **protease** domain of an endotheliase protein or a protein that is a catalytically active portion of it.
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for:
(1) (P1) where:
(a) the endotheliase **protease** domain portion of the protein consists essentially of a sequence of amino acids that comprises the **protease** domain or a catalytically active portion of it; and
(b) an endotheliase is an endothelial cell transmembrane **protease**;
(2) a substantially purified endotheliase 2 protein or a protein that is a catalytically active portion of it (P2);
(3) a nucleic acid (N2) that encodes an endotheliase protein or the **protease** domain of an endotheliase or catalytically active portion of the **protease** domain and that hybridizes under conditions of medium or high stringency along its full length to (N1);
(4) a vector (N3) comprising (N1) or (N2);
(5) a cell (I) comprising (N3);
(6) producing an endotheliase protein or endotheliase **protease** domain protein comprising:
(a) culturing (I) under conditions where the encoded endotheliase protein or endotheliase **protease** domain protein is expressed by the cell; and
(b) recovering the expressed protein;
(7) an antisense nucleic acid molecule that comprises at least 14 contiguous nucleotides or modified nucleotides complementary to the coding portion of (N1);
(8) an antibody (Ab) that specifically binds to a **protease** domain of (P1);
(9) a conjugate comprising:
(a) (P1);
(b) a targeting agent linked to the protein directly or via linker;
(10) a combination comprising:
(a) an inhibitor of the activity of an endotheliase or **protease** domain of it; and
(b) an another treatment or agent selected from anti-tumor and anti-angiogenic treatments or agents;
(11) a solid support comprising two or more (P1) linked to it directly or via a linker;
(12) identifying (M1) compounds that modulate the activity of an endotheliase comprising:
(a) contacting an endotheliase or **protease** domain of an endotheliase with a **substrate** proteolytically cleaved by the endotheliase, and either simultaneously, before or after, adding a test compound or plurality of it;
(b) measuring the amount of **substrate** cleaved in the presence of the test compound; and
(c) selecting compounds that change the amount cleaved compared to a control, where compounds that modulate the activity of the

endotheliase are identified;

(13) treating or preventing (M2) a disease or disorder associated with undesired and/or uncontrolled angiogenesis or neovascularization, in a mammal, comprising administering to a mammal an effective amount of an inhibitor of an endotheliase;

(14) identifying (M3) a compound that specifically binds to an endotheliase or a **protease** domain of it comprising:

(a) contacting the endotheliase or **protease** domain with a test compound or plurality of them under conditions conducive to binding; and

(b) identifying compounds that specifically bind to the endotheliase or to the **protease** domain of it; and

(15) a recombinant non-human animal, where an endogenous gene of an endotheliase has been deleted or inactivated by homologous recombination or insertional mutagenesis of the animal or an ancestor of it.

ACTIVITY - Cytostatic; Antidiabetic; Cardiant; Antiinflammatory; Antiulcer; Antirheumatic; Antiarthritic; Vulnerary; Nootropic; Opthalmic; Dermatological; Periodontal.

MECHANISM OF ACTION - Gene therapy; Antagonist.

USE - An endotheliase protein or protease domain of it is useful for the treatment or diagnosis of disorders associated with aberrant angiogenesis or undesired neovascularization. Inhibitors of endotheliase may be used to treat or prevent a disease or disorder associated with undesired and/or uncontrolled angiogenesis or neovascularization, in a mammal. The undesired angiogenesis is associated with disorders selected from solid neoplasm, vascular malformations and cardiovascular disorders, chronic inflammatory diseases and aberrant wound repairs, circulatory disorders, crest syndromes, dermatological disorders and ocular disorders. The vascular malformations and cardiovascular disorders are selected from angiofibroma, angiolipoma, atherosclerosis, restenosis/reperfusion injury, arterovenous malformations, hemangiomatosis and vascular adhesions, dyschondroplasia with vascular hamaromos (Fafucci's syndrome), hereditary hemorrhagic telandiectasia (Rendu-Osler-Weber syndrome) and Von Hipple Lindau syndrome. The chronic inflammatory diseases are selected from diabetes mellitus, hemophilliac joints, inflammatory bowel disease, nonhealing fractures, periodontitis, psoriasis, rheumatoid arthritis, venous stasis ulcers, granulations-burns, hypertrophic scars, liver cirrhosis, osteoradionecrosis, postoperative adhesion, pyogenic granuloma and systemic sclerosis, The circulatory disorders Taynaud's phenomenon, The crest syndromes are selected from systemic vasculitis, scleroderma, pyoderma gangrenosum, vasculopathy, venous, arterial ulcers, Struge-Weber syndrome, Port-wine stains, blue rubber bleb nevus syndrome, Klippel-Trenaunay-Weber syndrome and Osler-Weber-Rendu syndrome. The ocular disorders are selected from blindness caused by ocular neovascular disease, corneal graft neovascularization, macular degeneration, retinopathy of prematurity, retrolental fibroplasion and corneal neovascularization.

Dwg.0/1

L7 ANSWER 14 OF 34 WPIDS (C) 2002 THOMSON DERWENT
ACCESSION NUMBER: 2002-017124 [02] WPIDS
CROSS REFERENCE: 1999-095351 [08]; 2001-146289 [15]; 2001-367710 [38]; 2002-017125 [02]; 2002-017215 [02]; 2002-194904 [25]; 2002-340184 [37]

DOC. NO. NON-CPI: N2002-013777
DOC. NO. CPI: C2002-004765
TITLE: High through put screening of novel enzymes.
DERWENT CLASS: B04 D16 S03
INVENTOR(S): KELLER, M; SHORT, J M
PATENT ASSIGNEE(S): (RECO-N) RECOMBINANT BIOCATALYSIS INC
COUNTRY COUNT: 1
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
US 2001034031	A1	20011025	(200202)*		39

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
US 2001034031	A1 CIP of	US 1997-876276	19970616
	Cont of	US 1998-98206	19980616
	Div ex	US 2000-636778	20000811
		US 2001-848651	20010503

FILING DETAILS:

PATENT NO	KIND	PATENT NO
US 2001034031	A1 Cont of	US 6174673

PRIORITY APPLN. INFO: US 1998-98206 19980616; US 1997-876276 19970616; US 2000-636778 20000811; US 2001-848651 20010503

AN 2002-017124 [02] WPIDS

CR 1999-095351 [08]; 2001-146289 [15]; 2001-367710 [38]; 2002-017125 [02]; 2002-017215 [02]; 2002-194904 [25]; 2002-340184 [37]

AB US2001034031 A UPAB: 20020717

NOVELTY - A method of screening for an agent that modulates the interaction of a test protein linked to a DNA binding group and a test protein linked to a transcriptional activation group, by co-encapsulating the agent with the test proteins in a suitable microenvironment and determining the ability of the agent modulate the interaction of the test proteins, is new.

DETAILED DESCRIPTION - A method of screening for an agent that modulates the interaction of a first test protein linked to a DNA binding group and a second test protein linked to a transcriptional activation group, comprising co-encapsulating the agent with the first test protein and second test protein in a suitable microenvironment and determining the ability of the agent modulate the interaction of the first test protein linked to a DNA binding group with the second test protein covalently linked to a transcriptional activation group. The agent enhances or inhibits the expression of a detectable protein, and the enhancement or inhibition is detected by FACS analysis.

USE - The method is used for high throughput screening for novel enzymes.

ADVANTAGE - The method adapts traditional eukaryotic flow cytometry cell sorting systems for high through put screening of expression clones in **prokaryotes**. Expression libraries derived from DNA are screened rapidly for bioactivities of interest

utilizing fluorescence activated cell sorting. The libraries can contain greater than 108 members and can represent single organisms or can represent the genomes of over 100 different **microorganisms**, species or sub-species.
Dwg.0/18

L7 ANSWER 15 OF 34 WPIDS (C) 2002 THOMSON DERWENT
ACCESSION NUMBER: 2001-647984 [74] WPIDS
DOC. NO. NON-CPI: N2001-484211
DOC. NO. CPI: C2001-191105
TITLE: Identifying peptide-peptide interaction comprises identifying library encoded peptide as partner to target peptide if complex having first and second DNA binding domains fused to the peptides, binds to prokaryotic operator.
DERWENT CLASS: B04 D16 S03
INVENTOR(S): KODADEK, T J
PATENT ASSIGNEE(S): (KODA-I) KODADEK T J
COUNTRY COUNT: 1
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
US 2001029024	A1	20011011	(200174)*		33

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
US 2001029024	A1 Provisional	US 2000-182060P	20000211
		US 2001-780575	20010209

PRIORITY APPLN. INFO: US 2000-182060P 20000211; US 2001-780575 20010209
AN 2001-647984 [74] WPIDS
AB US2001029024 A UPAB: 20011217
NOVELTY - Identifying (M1) peptide-peptide interaction comprising contacting first, second fusion constructs (C1,C2) containing target peptide (T) or library encoded peptide (LEP) fused to first, second DNA binding domains (D1,D2) respectively, in prokaryotic host cell containing prokaryotic operator region (I), and determining binding of D1-D2 complex (X) to (I), where binding of (X) to (I) identifies LEP as binding partner for (T), is new.
DETAILED DESCRIPTION - (M1) comprises:
(a) providing a (C1) containing (T) fused to (D1);
(b) providing a (C2) containing a library encoded peptide (LEP) fused to (D2), where (D2) works as a complex with (D1) to facilitate binding of the complex to a prokaryotic operator region;
(c) contacting (C1) and (C2) in a prokaryotic host cell which contains the prokaryotic operator region which is operationally linked to coding region for one or more indicator polypeptides; and
(d) determining binding of (X) to the operator region, whereby binding of (X) to the operator region identifies the LEP as a binding partner for (T).
INDEPENDENT CLAIMS are also included for the following:
(1) a LEP selected by (M1); and
(2) a heterodimeric binding molecule (I) comprising a first and

second peptides that bind to (T), where at least one of the first and second peptides is a member of a peptide library; and a linker molecule connecting the first and second peptides such that the linking permits the first and second peptides to interact independently with (T).

USE - (M1) is useful for screening a peptide library for peptide-peptide interactions. LEP selected by (M1) is useful as capture probes for a specific target protein to which it binds, and for controlling the post translational modification of the proteins in a novel fashion, for example LEP identified by the method capable of recognizing a site of proteolysis or phosphorylation on target protein could protect that factor from chemical modification. The LEPs identified by the above method can be employed as epitope binding molecules (EBM). The high affinity epitope binding molecules can be used as capture agents in chip-based technologies. The identified LEPs are also used in a novel application termed **substrate**-directed inhibition by which **substrate** -targeted inhibitors are identified by the above mentioned method. The method involves fusing peptide sequence recognized by the enzyme (i.e., the **protease** cleavage site, kinase phosphorylation site, etc) to the repressor DNA-binding domain and a peptide library fused to another copy of repressor DNA-binding domain. The library-encoded peptides that bind to the target peptide with high affinity and specificity are identified by scanning the peptide library fused to repressor DNA-binding domain. The library-encoded peptides were then synthesized and evaluated as **substrate** -targeted inhibitors. These peptides may serve as lead compounds for the development of non-peptidic small molecule analogs, or may be used directly as drugs.

ADVANTAGE - The method provides a highly sensitive screening assay for the identification of peptide binding partners to virtually any peptide or polypeptide ligand. The method obviates the need to create epitope tagged versions of the native proteins for the purpose of immunoaffinity chromatography. Also many proteins would co-purify with associated partners by this method, that could be identified by mass spectrometry techniques. Thus the method provides an alternative to two-hybrid assay. The lambda repressor reconstitution system (the above described method, (M1), where lambda repressor is reconstituted by association of (D1) and (D2)) is sensitive to even low affinity interactions.

DESCRIPTION OF DRAWING(S) - The figure shows the schematic representation of the peptide library screening protocol.
Dwg.1A/9

L7 ANSWER 16 OF 34 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2001:219501 BIOSIS
DOCUMENT NUMBER: PREV200100219501
TITLE: ClpA mediates directional translocation of **substrate** proteins into the ClpP **protease**.
AUTHOR(S): Reid, Brian G.; Fenton, Wayne A.; Horwich, Arthur L.; Weber-Ban, Eilika U. (1)
CORPORATE SOURCE: (1) Eidgenossiche Technische Hochschule, Institut fur Molekularbiologie und Biophysik, Honggerberg HPK E3, CH-8093, Zurich: eilika@mol.biol.ethz.ch Switzerland
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (March 27, 2001) Vol. 98, No. 7, pp. 3768-3772. print.

ISSN: 0027-8424.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English

AB The intracellular degradation of many proteins is mediated in an ATP-dependent manner by large assemblies comprising a chaperone ring complex associated coaxially with a proteolytic cylinder, e.g., ClpAP, ClpXP, and HslUV in prokaryotes, and the 26S proteasome in eukaryotes. Recent studies of the chaperone ClpA indicate that it mediates ATP-dependent unfolding of **substrate** proteins and directs their ATP-dependent translocation into the ClpP **protease**. Because the axial passageway into the proteolytic chamber is narrow, it seems likely that unfolded **substrate** proteins are threaded from the chaperone into the **protease** , suggesting that translocation could be directional. We have investigated directionality in the ClpA/ClpP-mediated reaction by using two **substrate** proteins bearing the COOH-terminal ssrA recognition element, each labeled near the NH2 or COOH terminus with fluorescent probes. Time-dependent changes in both fluorescence anisotropy and fluorescence resonance energy transfer between donor fluorophores in the ClpP cavity and the **substrate** probes as acceptors were measured to monitor translocation of the **substrates** from ClpA into ClpP. We observed for both **substrates** that energy transfer occurs 2-4 s sooner with the COOH-terminally labeled molecules than with the NH2-terminally labeled ones, indicating that translocation is indeed directional, with the COOH terminus of the **substrate** protein entering ClpP first.

L7 ANSWER 17 OF 34 WPIDS (C) 2002 THOMSON DERWENT
ACCESSION NUMBER: 2000-303764 [26] WPIDS
DOC. NO. NON-CPI: N2000-226922
DOC. NO. CPI: C2000-092283
TITLE: DNA shuffling methods improve mycotoxin detoxification genes for use in agricultural and industrial processes to degrade mycotoxins.
DERWENT CLASS: C06 D16 P13
INVENTOR(S): SUBRAMANIAN, V
PATENT ASSIGNEE(S): (MAXY-N) MAXYGEN INC
COUNTRY COUNT: 88
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2000020573	A2	20000413	(200026)*	EN	68
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ TZ UG ZW					
W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZA ZW					
AU 9965107	A	20000426	(200036)		
EP 1119616	A2	20010801	(200144)	EN	
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI					
JP 2002526107	W	20020820	(200258)		95

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2000020573	A2	WO 1999-US23385	19991006
AU 9965107	A	AU 1999-65107	19991006
EP 1119616	A2	EP 1999-953091	19991006
		WO 1999-US23385	19991006
JP 2002526107	W	WO 1999-US23385	19991006
		JP 2000-574669	19991006

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 9965107	A	Based on	WO 200020573
EP 1119616	A2	Based on	WO 200020573
JP 2002526107	W	Based on	WO 200020573

PRIORITY APPLN. INFO: US 1998-103441P 19981007
AN 2000-303764 [26] WPIDS
AB WO 200020573 A UPAB: 20000531
NOVELTY - Use of DNA shuffling to generate new or improved mycotoxin detoxification (MD) genes, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the following:

(1) a method (A) for preparing a shuffled mycotoxin detoxification nucleic acid (MDNA) encoding a mycotoxin detoxification (MD) activity comprising:

(a) recombining several parental nucleic acids to produce one or more recombinant MDNA comprising a distinct or improved MD activity; and

(b) selecting the one or more recombinant MDNA for one or more encoded mycotoxin detoxification activity or for enhanced or reduced encoded polypeptide expression or stability;

(2) a nucleic acid (NA) encoding a MD activity prepared by the method of (1);

(3) a library of recombinant nucleic acids prepared using the method of (1) comprising one or more MD activities selected from inactivation or modification of a polyketide, aflatoxin, sterigmatocystin, trichothecene or fumonisin, an increased ability to chemically modify a mycotoxin, an increase in the range of mycotoxin **substrates** which a polypeptide encoded by the NA can modify, an increased expression level of a polypeptide encoded by the NA, a decrease in susceptibility of a polypeptide encoded by the NA to **protease** cleavage, high or low pH or temperature levels or a decrease in toxicity to a host cell of a polypeptide encoded by the selected NA;

(4) a library of recombinant nucleic acids comprising one or more monooxygenase activities prepared using the method of (3);

(5) method (A) with the additional steps of:

(i) recombining DNA from several cells that display MD activity with a library of DNA fragments, at least one of which undergoes recombination with a segment in a cellular DNA present in the cells to produce recombined cells, or recombining DNA between the cells that display MD activity to produce cells that have modified MD activity; or

(ii) recombining and screening the recombined or modified cells to produce further recombined cells that have evolved additionally

modified MD activity; and

(iii) repeating (i) and (ii) until the further recombined cells have acquired a desired MD activity;

(6) a further recombined cell which has acquired a desired MD activity prepared by the method of (5);

(7) method (A) with the additional steps of:

(i) recombining at least one distinct or improved NA with a further MDNA that is the same or different to one of the parental NAs to produce a library of recombinant MDNAs;

(ii) screening the library to identify at least one further distinct or improved recombinant MDNA that exhibits a further improvement or distinct property compared to the parent NAs; and optionally

(iii) repeating (i) and (ii) until the further distinct or improved recombinant NA shows additionally distinct or improved MD property;

(8) a plant transduced with the MDNA produced by the method of (1);

(9) a DNA shuffling mixture comprising at least 3 homologous DNAs each derived from a NA encoding a polypeptide or polypeptide fragment with MD activity; and

(10) a method (B) of increasing MD of a cell comprising carrying out whole genome shuffling of several genomic NAs in the cell and selecting for one or more MD activities.

USE - The mycotoxin detoxification genes produced by this method are used to provide enzymes which degrade mycotoxins in agricultural and industrial processes. The enzymes are used to transform mycotoxins produced by fungal pathogens in crops into compounds which are not toxic to plants, animals and humans, alleviating food pollution and the costs involved with detecting and treating contamination of crops.

ADVANTAGE - The genes produced have superior properties compared to naturally occurring mycotoxin detoxification genes. DNA shuffling optimizes the activity of the genes without requiring an understanding of the mechanism of the activity being optimized.

Dwg.0/0

L7 ANSWER 18 OF 34 WPIDS (C) 2002 THOMSON DERWENT
ACCESSION NUMBER: 2000-533868 [49] WPIDS
DOC. NO. CPI: C2000-159308
TITLE: Host cell, useful e.g. as bioreactor for production of poly(hydroxyalkanoate), containing two or more recombinant polypeptides, with at least one in carrier-bound form.
DERWENT CLASS: B04 D16
INVENTOR(S): LUBITZ, W
PATENT ASSIGNEE(S): (LUBI-I) LUBITZ W
COUNTRY COUNT: 91
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
DE 19903345	A1	20000803	(200049)*		25
WO 2000044878	A1	20000803	(200049)	GE	

RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ TZ UG ZW

W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK DM EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ

LC LK LR LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU
SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW
AU 2000026675 A 20000818 (200057)
EP 1144590 A1 20011017 (200169) GE
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK
NL PT RO SE SI
CN 1345370 A 20020417 (200248)

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
DE 19903345	A1	DE 1999-19903345	19990128
WO 2000044878	A1	WO 2000-EP686	20000128
AU 2000026675	A	AU 2000-26675	20000128
EP 1144590	A1	EP 2000-904978	20000128
		WO 2000-EP686	20000128
CN 1345370	A	CN 2000-802985	20000128

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 2000026675	A	Based on	WO 200044878
EP 1144590	A1	Based on	WO 200044878

PRIORITY APPLN. INFO: DE 1999-19903345 19990128
AN 2000-533868 [49] WPIDS
AB DE 19903345 A UPAB: 20001006
NOVELTY - Host cell (A) comprising at least two functional recombinant polypeptides (I), at least one being in carrier bound form, is new.
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:
(1) recombinant bacterial ghosts (B) produced from (A); and
(2) method for preparing (A).
ACTIVITY - Antibacterial.
MECHANISM OF ACTION - Vaccine
No biological data given.
USE - (A), or, where bacterial, their ghosts (B), are useful as vaccines or adjuvants (specifically for presentation of immunogenic epitopes of pathogens or autologous immunostimulatory polypeptides, e.g. cytokines), or preferably, as enzyme reactors for performing a cascade of reactions, specifically synthesis of poly(hydroxyalkanoate).
ADVANTAGE - Localization of individual (I), specifically enzymes, in separate cellular compartments avoids adverse reactions between products and **substrates**, when being used as bioreactors. (I) can be produced in carrier-bound form without loss of function.
Dwg.0/2

L7 ANSWER 19 OF 34 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2001:81340 BIOSIS
DOCUMENT NUMBER: PREV200100081340
TITLE: Mutational studies on HslU and its docking mode with HslV.
AUTHOR(S): Song, Hyun Kyu; Hartmann, Claudia; Ramachandran,

Ravishankar; Bochtler, Matthias; Behrendt, Raymond; Moroder, Luis; Huber, Robert (1)
CORPORATE SOURCE: (1) Abteilung Strukturforschung, Max-Planck-Institut fuer Biochemie, Am Klopferspitz 18a, D-82152, Planegg-Martinsried: huber@biochem.mpg.de Germany
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (December 19, 2000) Vol. 97, No. 26, pp. 14103-14108. print.
ISSN: 0027-8424.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English
AB HslVU is an ATP-dependent prokaryotic **protease** complex. Despite detailed crystal and molecular structure determinations of free HslV and HslU, the mechanism of ATP-dependent peptide and protein hydrolysis remained unclear, mainly because the productive complex of HslV and HslU could not be unambiguously identified from the crystal data. In the crystalline complex, the I domains of HslU interact with HslV. Observations based on electron microscopy data were interpreted in the light of the crystal structure to indicate an alternative mode of association with the intermediate domains away from HslV. By generation and analysis of two dozen HslU mutants, we find that the amidolytic and caseinolytic activities of HslVU are quite robust to mutations on both alternative docking surfaces on HslU. In contrast, HslVU activity against the maltose-binding protein-SulA fusion protein depends on the presence of the I domain and is also sensitive to mutations in the N-terminal and C-terminal domains of HslU. Mutational studies around the hexameric pore of HslU seem to show that it is involved in the recognition/translocation of maltose-binding protein-SulA but not of chromogenic small **substrates** and casein. ATP-binding site mutations, among other things, confirm the essential role of the "sensor arginine" (R393) and the "arginine finger" (R325) in the ATPase action of HslU and demonstrate an important role for E321. Additionally, we report a better refined structure of the HslVU complex crystallized along with resorufin-labeled casein.

L7 ANSWER 20 OF 34 MEDLINE DUPLICATE 2
ACCESSION NUMBER: 2000191959 MEDLINE
DOCUMENT NUMBER: 20191959 PubMed ID: 10725424
TITLE: Role of Ser-652 and Lys-692 in the **protease** activity of infectious bursal disease virus VP4 and identification of its **substrate** cleavage sites.
AUTHOR: Lejal N; Da Costa B; Huet J C; Delmas B
CORPORATE SOURCE: Unite de Virologie et Immunologie moleculaires and Unite de Biochimie et Structure des proteines, Institut National de la Recherche Agronomique, F-78350 Jouy-en-Josas, France.
SOURCE: JOURNAL OF GENERAL VIROLOGY, (2000 Apr) 81 (Pt 4) 983-92.
Journal code: 0077340. ISSN: 0022-1317.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200005
ENTRY DATE: Entered STN: 20000525

Last Updated on STN: 20020212
Entered Medline: 20000518

AB The polyprotein of infectious bursal disease virus (IBDV), an avian birnavirus, is processed by the viral **protease**, VP4. Previous data obtained on the VP4 of infectious pancreatic necrosis virus (IPNV), a fish birnavirus, and comparative sequence analysis between IBDV and IPNV suggest that VP4 is an unusual eukaryotic serine **protease** that shares properties with **prokaryotic** leader peptidases and other **bacterial** peptidases. IBDV VP4 is predicted to utilize a serine-lysine catalytic dyad. Replacement of the members of the predicted catalytic dyad (Ser-652 and Lys-692) confirmed their indispensability. The two cleavage sites at the pVP2-VP4 and VP4-VP3 junctions were identified by N-terminal sequencing and probed by site-directed mutagenesis. Several additional candidate cleavage sites were identified in the C-terminal domain of pVP2 and tested by cumulative site-directed mutagenesis and expression of the mutant polyproteins. The results suggest that VP4 cleaves multiple (Thr/Ala)-X-Ala downward arrowAla motifs. A trans activity of the VP4 **protease** of IBDV, and also IPNV VP4 **protease**, was demonstrated by co-expression of VP4 and a polypeptide **substrate** in Escherichia coli. For both **proteases**, cleavage specificity was identical in the cis- and trans-activity assays. An attempt was made to determine whether VP4 **proteases** of IBDV and IPNV were able to cleave heterologous **substrates**. In each case, no cleavage was observed with heterologous combinations. These results on the IBDV VP4 confirm and extend our previous characterization of the IPNV VP4, delineating the birnavirus **protease** as a new type of viral serine **protease**.

L7 ANSWER 21 OF 34 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2000:179818 BIOSIS
DOCUMENT NUMBER: PREV200000179818
TITLE: Intracellular proteolysis: Signals of selective protein degradation.
AUTHOR(S): Starkova, N. N.; Koroleva, E. P.; Rotanova, T. V. (1)
CORPORATE SOURCE: (1) Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, ul. Miklukho-Maklaya 16/10, GSP-7, Moscow, 117871 Russia
SOURCE: Bioorganicheskaya Khimiya, (Feb., 2000) Vol. 26, No. 2, pp. 83-96.
ISSN: 0132-3423.
DOCUMENT TYPE: Article
LANGUAGE: Russian
SUMMARY LANGUAGE: English; Russian

AB Selective proteolysis is one of the mechanisms for the maintenance of cell homeostasis via rapid degradation of defective polypeptides and certain short-lived regulatory proteins. In prokaryotic cells, high-molecular-mass oligomeric ATP-dependent **proteases** are responsible for selective protein degradation. In eukaryotes, most polypeptides are attacked by the multicatalytic 26S proteasome, and the degradation of the majority of **substrates** involves their preliminary modification with the protein ubiquitin. The proteins undergoing the selective proteolysis often contain specific degradation signals necessary for their recognition by the corresponding **proteases**.

L7 ANSWER 22 OF 34 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1999:496440 BIOSIS
DOCUMENT NUMBER: PREV199900496440
TITLE: Chaperone rings in protein folding and degradation.
AUTHOR(S): Horwich, Arthur L. (1); Weber-Ban, Eilika U.; Finley, Daniel
CORPORATE SOURCE: (1) Department of Genetics, Yale School of Medicine, New Haven, CT, 06510 USA
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (Sept. 28, 1999) Vol. 96, No. 20, pp. 11033-11040.
ISSN: 0027-8424.
DOCUMENT TYPE: General Review
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Chaperone rings play a vital role in the opposing ATP-mediated processes of folding and degradation of many cellular proteins, but the mechanisms by which they assist these life and death actions are only beginning to be understood. Ring structures present an advantage to both processes, providing for compartmentalization of the **substrate** protein inside a central cavity in which multivalent, potentially cooperative interactions can take place between the **substrate** and a high local concentration of binding sites, while access of other proteins to the cavity is restricted sterically. Such restriction prevents outside interference that could lead to nonproductive fates of the **substrate** protein while it is present in non-native form, such as aggregation. At the step of recognition, chaperone rings recognize different motifs in their **substrates**, exposed hydrophobicity in the case of protein-folding chaperonins, and specific "tag" sequences in at least some cases of the proteolytic chaperones. For both folding and proteolytic complexes, ATP directs conformational changes in the chaperone rings that govern release of the bound polypeptide. In the case of chaperonins, ATP enables a released protein to pursue the native state in a sequestered hydrophilic folding chamber, and, in the case of the **proteases**, the released polypeptide is translocated into a degradation chamber. These divergent fates are at least partly governed by very different cooperating components that associate with the chaperone rings: that is, cochaperonin rings on one hand and proteolytic ring assemblies on the other. Here we review the structures and mechanisms of the two types of chaperone ring system.

L7 ANSWER 23 OF 34 MEDLINE DUPLICATE 3
ACCESSION NUMBER: 2000427090 MEDLINE
DOCUMENT NUMBER: 20395196 PubMed ID: 10941781
TITLE: Bacterial signals and antagonists: the interaction between bacteria and higher organisms.
AUTHOR: Rice S A; Givskov M; Steinberg P; Kjelleberg S
CORPORATE SOURCE: The School of Microbiology and Immunology, The University of New South Wales, Sydney, Australia.
SOURCE: JOURNAL OF MOLECULAR MICROBIOLOGY AND BIOTECHNOLOGY, (1999 Aug) 1 (1) 23-31. Ref: 63
Journal code: 100892561. ISSN: 1464-1801.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)

LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200009
ENTRY DATE: Entered STN: 20000922
Last Updated on STN: 20000922
Entered Medline: 20000912

AB It is now well established that **bacteria** communicate through the secretion and uptake of small diffusable molecules. These chemical cues, or signals, are often used by **bacteria** to coordinate phenotypic expression and this mechanism of regulation presumably provides them with a competitive advantage in their natural environment. Examples of coordinated behaviors of marine **bacteria** which are regulated by signals include swarming and exoprotease production, which are important for niche colonisation or nutrient acquisition (e.g. **protease** breakdown of **substrate**). While the current focus on **bacterial** signalling centers on N-Acylated homoserine lactones, the quorum sensing signals of gram-negative **bacteria**, these are not the only types of signals used by **bacteria**. Indeed, there appears to be many other types of signals produced by **bacteria** and it also appears that a **bacterium** may use multiple classes of signals for phenotypic regulation. Recent work in the area of marine microbial ecology has led to the observation that some marine eukaryotes secrete their own signals which compete with the **bacterial** signals and thus inhibit the expression of **bacterial** signalling phenotypes. This type of molecular mimicry has been well characterised for the interaction of marine **prokaryotes** with the red alga, Delisea pulchra.

L7 ANSWER 24 OF 34 WPIDS (C) 2002 THOMSON DERWENT
ACCESSION NUMBER: 1999-095351 [08] WPIDS
CROSS REFERENCE: 2001-146289 [15]; 2001-367710 [38]; 2002-017124 [02]; 2002-017125 [02]; 2002-017215 [02]; 2002-194904 [25]; 2002-340184 [37]
DOC. NO. CPI: C1999-028097
TITLE: High throughput screening of **prokaryotic** genomic DNA for novel enzymes - enables identification of enzymes from uncultured **micro-organisms** derived from environmental samples, useful industrially as catalysts.
DERWENT CLASS: B04 D16 J04
INVENTOR(S): KELLER, M; SHORT, J M
PATENT ASSIGNEE(S): (DIVE-N) DIVERSA CORP
COUNTRY COUNT: 23
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 9858085	A1	19981223	(199908)*	EN	95
RW: AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE					
W: AU CA JP US					
AU 9881502	A	19990104	(199921)		
EP 1009858	A1	20000621	(200033)	EN	
R: AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE					
JP 2002505590	W	20020219	(200216)		89
AU 2002035649	A	20020613	(200251)#		

AU 749587 B 20020627 (200254)

APPLICATION DETAILS:

PATENT NO	KIND		APPLICATION	DATE
WO 9858085	A1		WO 1998-US12674	19980616
AU 9881502	A		AU 1998-81502	19980616
EP 1009858	A1		EP 1998-931354	19980616
			WO 1998-US12674	19980616
JP 2002505590	W		WO 1998-US12674	19980616
			JP 1999-504782	19980616
AU 2002035649	A		AU 2002-35649	20020424
		Div ex	AU 1998-81502	
AU 749587	B		AU 1998-81502	19980616

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 9881502	A	Based on	WO 9858085
EP 1009858	A1	Based on	WO 9858085
JP 2002505590	W	Based on	WO 9858085
AU 2002035649	A	Div ex	AU 749587
AU 749587	B	Previous Publ.	AU 9881502
		Based on	WO 9858085

PRIORITY APPLN. INFO: US 1997-876276 19970616; AU 2002-35649 20020424

AN 1999-095351 [08] WPIDS

CR 2001-146289 [15]; 2001-367710 [38]; 2002-017124 [02]; 2002-017125 [02]; 2002-017215 [02]; 2002-194904 [25]; 2002-340184 [37]

AB WO 9858085 A UPAB: 20020823

A method of high throughput screening of prokaryotic genomic DNA samples to identify one or more enzymes encoded by the DNA comprise: (a) generating a multispecific, prokaryotic expression library; (b) inserting bioactive **substrates** into samples of library; (c) screening samples with fluorescent analyser that detects bioactive fluorescence; and (d) separating positive samples, in which DNA sequence encodes enzyme catalysing bioactive **substrate** of (b). Also claimed are assays optionally utilising enzymes/DNA identified as above and requiring co-encapsulation: (1) screening for an agent (optionally enzyme e.g. derived as above or small molecule) modulating activity of a target cell component in a recombinant cell expressing the component and a detectable marker, by co-encapsulating agent in a micro-environment with recombinant cell and detecting effect of agent on activity of cell component; (2) screening for an agent (optionally an enzyme e.g. derived as above or small molecule) modulating interaction of test protein linked to a DNA binding moiety and a second test protein linked to a transcriptional activation moiety, by co-encapsulating agent with the two test proteins in a micro-environment and determining ability of agent to modulate interaction of test proteins by detecting enhancement/inhibition of expression of a detectable protein by fluorescence-activated cell sorting (FACS) analysis; and (3) enriching for target DNA sequences containing at least partial coding region for at least one specified activity in a DNA sample, by (i) co-encapsulating in a

micro-environment a mixture of target DNA from several organisms with a mixture of DNA probes comprising detectable marker and at least one portion of DNA sequence encoding an enzyme with specified activity (e.g. derived as above); (ii) incubating under conditions suitable for hybridisation of complementary sequences; and (iii) screening for specified activity.

USE - The method can be used to identify enzymes such as lipases, esterases, **proteases** etc. (claimed) useful industrially as catalysts. It provides high throughput screening, so is especially useful to identify such enzymes from uncultured micro-organisms derived from environmental samples, where screening of several million clones may be required to cover the genomic biodiversity. It is useful to screen expression libraries containing extremophiles (e.g. hyperthermophiles, psychrophiles and especially thermophiles; claimed), since these organisms may provide enzymes which can perform under demanding industrial conditions e.g. of high temperature. It can also be used to derive potential enzymes useful in the assays of (1)-(3) e.g. lipases, esterases, **proteases** etc. useful in methods (1) and (2) (claimed) which e.g. affect the action of transducing proteins (e.g. G-proteins; claimed) in method (1) or enhance or inhibit ligand/receptor interactions enabling drug screening in method (2). Method (3) can be used to enrich a population of clones for target sequences coding for specified activities, especially in uncultured micro-organisms (claimed) from environmental samples (especially terrestrial, marine and/or airborne micro-organisms (claimed) and particularly extremophiles as above (claimed))

ADVANTAGE - Method allows high throughput screening (30-200 million clones/hr) so enables culture-independent screening of environmental samples, and thus access to largely untapped source of novel enzymes.

Dwg.0/18

L7 ANSWER 25 OF 34 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
DUPLICATE 4

ACCESSION NUMBER: 1997:506675 BIOSIS
DOCUMENT NUMBER: PREV199799805878
TITLE: Functional expression of a tobacco gene related to the serine hydrolase family esterase activity towards short-chain dinitrophenyl acylesters.
AUTHOR(S): Baudouin, Emmanuel; Charpenteau, Martine; Roby, Dominique; Marco, Yves; Ranjeva, Raoul; Ranty, Benoit (1)
CORPORATE SOURCE: (1) Lab. Signaux Messages Cellulaires chez les Vegetaux, UMR 5546 CNRS/Univ. P. Sabatier, Bat. IVR1, Univ. Paul Sabatier, 118 route de Narbonne, F-31062 Toulouse Cedex 4 France
SOURCE: European Journal of Biochemistry, (1997) Vol. 248, No. 3, pp. 700-706.
ISSN: 0014-2956.
DOCUMENT TYPE: Article
LANGUAGE: English

AB We have recently reported the isolation of a tobacco gene, hsr 203J, whose transcripts accumulate during the hypersensitive reaction, a plant response associated with resistance to **pathogens**. We present and discuss here some structural and biochemical properties of the gene product. Nucleotide sequence analysis has shown that the hsr 203J gene contains an open reading frame coding for a

polypeptide of 335 amino acids. The predicted amino acid sequence contains the GXSXG motif characteristic of serine hydrolases, and displays limited but significant similarity to lipases and esterases of **prokaryotic** origin. The hsr 203J gene was expressed in Escherichia coli, and the recombinant protein, purified to near homogeneity, was able to degrade p-nitrophenylbutyrate, a general **substrate** for carboxylesterases. The enzyme was unable to hydrolyze lipids, and was active on short-chain acyl esters only. The hydrolytic activity was abolished by diisopropyl fluorophosphate and a derivative of isocoumarin, as expected for a member of the serine hydrolase family. Sequence similarities between the tobacco esterase and expressed sequence tags in databases suggest the existence of members of this enzyme family in various plant species.

L7 ANSWER 26 OF 34 SCISEARCH COPYRIGHT 2002 ISI (R)
ACCESSION NUMBER: 96:916384 SCISEARCH
THE GENUINE ARTICLE: VX168
TITLE: Programmed cell death in bacteria
AUTHOR: Chaloupka J (Reprint); Vinter V
CORPORATE SOURCE: ACAD SCI CZECH REPUBL, DEPT CELL & MOL MICROBIOL, INST MICROBIOL, PRAGUE 14220 4, CZECH REPUBLIC (Reprint)
COUNTRY OF AUTHOR: CZECH REPUBLIC
SOURCE: FOLIA MICROBIOLOGICA, (NOV-DEC 1996) Vol. 41, No. 6, pp. 451-464.
Publisher: FOLIA MICROBIOLOGICA, INST MICROBIOLOGY, VIDENSKA 1083, PRAGUE 4, CZECH REPUBLIC 142 20.
ISSN: 0015-5632.
DOCUMENT TYPE: General Review; Journal
FILE SEGMENT: LIFE; AGRI
LANGUAGE: English
REFERENCE COUNT: 135

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Programmed death (PDC) of individual cells is a genetically controlled biological process related to the development of multicellular organisms. It proceeds in most cases as apoptosis characterized by DNA degradation and breakdown of dying cells to apoptotic bodies, and ending by their phagocytosis by macrophages or by the surrounding tissue. Unlike apoptosis, necrosis is a genetically unregulated sudden death of a group of cells caused by a severe damage of membranes and other cell components. In **bacteria**, programmed cell death is mostly related to population development. This holds mainly for sporulation of bacilli where the process is best understood at the morphological, physiological and genetic level. Sporulation of bacilli begins by an asymmetric division of the nongrowing cell into two parts - the mother and the forespore compartment, whose fate is different. Whereas the smaller compartment develops into the spore, the function of the larger is twofold. It participates in the spore development mainly by forming spore coats but it also synthesizes or activates the autolytic apparatus which lyzes the sporangium cell wall at the end of the process. Some phases of the development of myxobacteria and streptomycetes also have characteristic features of programmed death. Unlike sporulation of bacilli, the autolysis of a portion of population of myxobacteria or hyphae of streptomycetes proceeds in the middle of their developmental cycle. Extensive turnover of cell membranes in growing mqxobacteria results in the formation of a fatty acid mixture - the autocide - which kills a

smaller or greater portion of the myxobacterial population. The dead cells are digested by extracellular enzymes released by myxobacteria and the digest is used as nutrient for completion of the developmental cycle of the remaining living population. Similar events take place also during the formation of aerial mycelium in streptomycetes. Here the autolysis of a portion of vegetative (**substrate**) mycelium supplies amino acids for the formation of aerial mycelium. The recently discovered programmed death of plasmid-free descendants of a plasmid-bearing population of different **bacteria** is based on the loss of control of toxin activity by its antidote. Both substances are encoded by plasmid DNA and the loss of the plasmid results in an ''enforced suicide'' of the host cell because the effective concentration of the antidote decreases more rapidly than that of the toxin. The mechanisms of this suicide can vary. In addition to the above mentioned kinds of programmed death, other events of developmentally regulated death of **prokaryotes** probably exist. Some **bacteria** contain ''death genes'' in their chromosome which trigger cell death at the onset of the stationary phase. The physiological function of this kind of suicide is not known. However, most nonsporulating **bacteria** developed a strategy of surviving at the nongrowing stage by transforming the growing cell to a more resistant dormant (cryptobiological) form.

L7 ANSWER 27 OF 34 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
DUPLICATE 5

ACCESSION NUMBER: 1996:59226 BIOSIS
DOCUMENT NUMBER: PREV199698631361
TITLE: Cloning and expression of trypsin-like enzyme from Streptomyces fradiae for comparative analysis of functional regions of Streptomyces and mammalian trypsins.
AUTHOR(S): Katoh, Takaaki (1); Kikuchi, Norihisa; Nagata, Kiyoshi; Yoshida, Nobuo
CORPORATE SOURCE: (1) Shionogi Res. Labs., Shionogi and Co., Ltd., 12-4, Sagisu 5-chome, Fukushima-ku, Osaka 553 Japan
SOURCE: Journal of Fermentation and Bioengineering, (1995) Vol. 80, No. 5, pp. 440-445. ISSN: 0922-338X.
DOCUMENT TYPE: Article
LANGUAGE: English

AB The trypsin-like enzyme from Streptomyces fradiae (SFT) is one of the extracellular **proteases** secreted by gram-positive **bacteria**. Since the primary structure of SFT is still unknown, a gene encoding SFT was cloned from a S. fradiae genomic library using an amplified polymerase chain reaction product within the SFT gene as a probe. The nucleotide sequence of the cloned fragment revealed that the gene encoded an open reading frame with 259 amino acids. The 38 N-terminal amino acids resemble a typical **prokaryotic** signal peptide, but the predicted signal sequence cleavage site suggests the existence of a very short, four-amino-acid prosequence. The mature SFT consists of 221 amino acids with a molecular weight of 22900. SFT was expressed in Streptomyces lividans 1326 using pIJ702 as a vector, and the secreted protein was purified from culture supernatant by soybean trypsin inhibitor-affinity chromatography. The N-terminal amino acid sequence and molecular weight of the protein were identical to those of natural SFT, indicating correct processing by S. lividans 1326.

Also, the amino acid composition of the recombinant SFT agreed with that of natural SFT and that deduced from the nucleotide sequence. Comparison of the amino acid sequence of SFT and other trypsins of microbial and mammalian origins revealed that SFT exhibits 85% identity to Streptomyces griseus trypsin (SGT), but low identity to Saccharopolyspora erythraea trypsin (SET) (38%) and to bovine trypsin (35%). The sequence alignment shows that the catalytic triad, the **substrate**-binding site and six cysteine residues are highly conserved. We found amino acid substitutions between SFT and SGT in regions involved in **substrate** specificity and sequence differences between Streptomyces and mammalian trypsins in **substrate**-binding regions.

L7 ANSWER 28 OF 34 MEDLINE DUPLICATE 6
ACCESSION NUMBER: 94329573 MEDLINE
DOCUMENT NUMBER: 94329573 PubMed ID: 8052636
TITLE: Protein synthesis elongation factor EF-1 alpha is essential for ubiquitin-dependent degradation of certain N alpha-acetylated proteins and may be substituted for by the bacterial elongation factor EF-Tu.
AUTHOR: Gonen H; Smith C E; Siegel N R; Kahana C; Merrick W C; Chakraburtty K; Schwartz A L; Ciechanover A
CORPORATE SOURCE: Department of Biochemistry, Faculty of Medicine, Technion-Israel Institute of Technology, Haifa.
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1994 Aug 2) 91 (16) 7648-52.
Journal code: 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199409
ENTRY DATE: Entered STN: 19940914
Last Updated on STN: 20000303
Entered Medline: 19940902

AB Targeting of different cellular proteins for conjugation and subsequent degradation via the ubiquitin pathway involves diverse recognition signals and distinct enzymatic factors. A few proteins are recognized via their N-terminal amino acid residue and conjugated by a ubiquitin-protein ligase that recognizes this residue. Most **substrates**, including the N alpha-acetylated proteins that constitute the vast majority of cellular proteins, are targeted by different signals and are recognized by yet unknown ligases. We have previously shown that degradation of N-terminally blocked proteins requires a specific factor, designated FH, and that the factor acts along with the 26S **protease** complex to degrade ubiquitin-conjugated proteins. Here, we demonstrate that FH is the protein synthesis elongation factor EF-1 alpha. (a) Partial sequence analysis reveals 100% identity to EF-1 alpha. (b) Like EF-1 alpha, FH binds to immobilized GTP (or GDP) and can be purified in one step using the corresponding nucleotide for elution. (c) Guanine nucleotides that bind to EF-1 alpha protect the ubiquitin system-related activity of FH from heat inactivation, and nucleotides that do not bind do not exert this effect. (d) EF-Tu, the homologous **bacterial** elongation factor, can substitute for FH/EF-1 alpha in the proteolytic system. This last finding is of

particular interest since the ubiquitin system has not been identified in **prokaryotes**. The activities of both EF-1 alpha and EF-Tu are strongly and specifically inhibited by ubiquitin-aldehyde, a specific inhibitor of ubiquitin isopeptidases. It appears, therefore, that EF-1 alpha may be involved in releasing ubiquitin from multiubiquitin chains, thus rendering the conjugates susceptible to the action of the 26S **protease** complex.

L7 ANSWER 29 OF 34 SCISEARCH COPYRIGHT 2002 ISI (R)
ACCESSION NUMBER: 94:5828 SCISEARCH
THE GENUINE ARTICLE: MN352
TITLE: EVOLUTIONARY DIVERGENCE AND CONSERVATION OF TRYPSIN
AUTHOR: RYPNIEWSKI W R (Reprint); PERRAKIS A; VORGIAS C E; WILSON K S
CORPORATE SOURCE: DESY, EUROPEAN MOLEC BIOL LAB, NOTKESTR 85, D-22603 HAMBURG, GERMANY (Reprint)
COUNTRY OF AUTHOR: GERMANY
SOURCE: PROTEIN ENGINEERING, (JAN 1994) Vol. 7, No. 1, pp. 57-64.
ISSN: 0269-2139.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 42
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The trypsin sequences currently available in the data banks have been collected and aligned using first the amino acid sequence homology and, subsequently, the superposed crystal structures of trypsins from the cow, the **bacterium** Streptomyces griseus and the fungus Fusarium oxysporum. The phylogenetic tree constructed according to this multiple alignment is consistent with a continuous evolutionary divergence of trypsin from a common ancestor of both **prokaryotes** and eukaryotes. Comparison of crystal structures reveals a strict conservation of secondary structure. Similarly, in the alignment of all the sequences, insertions and deletions occur only in regions corresponding to loops between the secondary structure elements in the known crystal structures. The conserved residues cluster around the active site. Almost all conserved residues can be associated with one of the basic functional features of the protein: zymogen activation, catalysis and **substrate** specificity. In contrast, the residues of the hydrophobic core of the protein and the calcium ion binding sites are generally not conserved. The conserved features of trypsin and the nature of the conservation are discussed in detail.

L7 ANSWER 30 OF 34 MEDLINE DUPLICATE 7
ACCESSION NUMBER: 92331613 MEDLINE
DOCUMENT NUMBER: 92331613 PubMed ID: 1628623
TITLE: Activation of mammalian DNA methyltransferase by cleavage of a Zn binding regulatory domain.
AUTHOR: Bestor T H
CORPORATE SOURCE: Department of Anatomy and Cellular Biology, Harvard Medical School, Boston, MA 02115.
CONTRACT NUMBER: GM43565 (NIGMS)
SOURCE: EMBO JOURNAL, (1992 Jul) 11 (7) 2611-7.
Journal code: 8208664. ISSN: 0261-4189.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199208
ENTRY DATE: Entered STN: 19920904
Last Updated on STN: 19970203
Entered Medline: 19920818

AB Mammalian DNA (cytosine-5) methyltransferase contains a C-terminal domain that is closely related to **bacterial** cytosine-5 restriction methyltransferase. This methyltransferase domain is linked to a large N-terminal domain. It is shown here that the N-terminal domain contains a Zn binding site and that the N- and C-terminal domains can be separated by cleavage with trypsin or Staphylococcus aureus **protease** V8; the **protease** V8 cleavage site was determined by Edman degradation to lie 10 residues C-terminal of the run of alternating lysyl and glycyl residues which joins the two domains and six residues N-terminal of the first sequence motif conserved between the mammalian and **bacterial** cytosine methyltransferases. While the intact enzyme had little activity on unmethylated DNA **substrates**, cleavage between the domains caused a large stimulation of the initial velocity of methylation of unmethylated DNA without substantial change in the rate of methylation of hemimethylated DNA. These findings indicate that the N-terminal domain of DNA methyltransferase ensures the clonal propagation of methylation patterns through inhibition of the de novo activity of the C-terminal domain. Mammalian DNA methyltransferase is likely to have arisen via fusion of a **prokaryotic**-like restriction methyltransferase and an unrelated DNA binding protein. Stimulation of the de novo activity of DNA methyltransferase by proteolytic cleavage in vivo may contribute to the process of ectopic methylation observed in the DNA of aging animals, tumors and in lines of cultured cells.

L7 ANSWER 31 OF 34 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1990:199220 BIOSIS
DOCUMENT NUMBER: BA89:105891
TITLE: BIOCHEMICAL MECHANISM OF KERATIN DEGRADATION BY THE ACTINOMYCETE STREPTOMYCES-FRADIAE AND THE FUNGUS MICROSPORUM-GYPSEUM A COMPARISON.
AUTHOR(S): KUNERT J
CORPORATE SOURCE: DEP. BIOL., FAC. MED., PALACKY UNIV., 775 15 OLOMOUC, CZECHOSLOVAKIA.
SOURCE: J BASIC MICROBIOL, (1989) 29 (9), 597-604.
CODEN: JBMIEQ. ISSN: 0233-111X.
FILE SEGMENT: BA; OLD
LANGUAGE: English

AB Two keratinolytic organisms, the **procaryote** Streptomyces fradiae and the fungus Microsporum gypseum, were cultured on sterile sheep's wool in a mineral solution. The loss in **substrate** was recorded and the degradation products in the cultivation fluid were analyzed. In M. gypseum the key reaction was the cleaving of the **substrate** disulfide bridges by means of sulfite excreted into the medium. Keratin denatured by "sulfitolysis" was further attacked by extracellular **proteases**. A typical finding was the accumulation of peptides containing S-sulfocysteine, the product of sulfitolysis of cystine. The overall excess of sulfur was removed by oxidation to sulfite and to sulfate, which was the main and final product. In S. fradiae the degradation was faster.

The results did not prove that sulfite formed and the concentration of sulfate in the medium remained negligible. Neither could cysteine desulfhydration and hydrogen sulfide excretion be demonstrated. The medium was found to contain relatively high concentrations of sulfhydryl compounds, evidently cysteine-containing peptides. Therefore, in this **microorganism**, keratin was most likely denatured by the direct reduction of cystine bridges. The main product of the elimination of excess sulfur was inorganic thiosulfate, which accumulated in the medium.

L7 ANSWER 32 OF 34 MEDLINE DUPLICATE 8
ACCESSION NUMBER: 87194892 MEDLINE
DOCUMENT NUMBER: 87194892 PubMed ID: 2952653
TITLE: Isolation and characterization of a fibronectin receptor from Staphylococcus aureus.
AUTHOR: Froman G; Switalski L M; Speziale P; Hook M
CONTRACT NUMBER: AI 20624 (NIAID)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1987 May 15) 262 (14) 6564-71.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198706
ENTRY DATE: Entered STN: 19900303
Last Updated on STN: 19970203
Entered Medline: 19870619

AB Attachment of **bacteria** to the host tissue is considered a first step in the development of many infections. Previous studies have shown that fibronectin, a protein shown to mediate **substrate** adhesion of eukaryotic cells, also binds to some pathogenic **bacteria** and mediates the tissue adherence of these **prokaryotes**. In the present communication, we report on the isolation and characterization of a fibronectin receptor from Staphylococcus aureus strain Newman. A 210-kDa fibronectin binding protein was isolated from a **bacterial** lysate by affinity chromatography followed by gel chromatography. Additional smaller peptides with fibronectin binding properties were also obtained. These peptides seem to represent degradation products of the large receptor protein since the former dominated when the purification was carried out in the absence of **protease** inhibitors. Furthermore, degradation of the purified receptor protein by staphylococcal V8 **protease** generated a large number of peptides that retained fibronectin binding activity. This observation also suggests that the large receptor protein contains several binding sites for fibronectin, and analysis of the binding of the 29-kDa amino-terminal fibronectin fragment to the 210-kDa receptor adsorbed in microtiter wells suggests that one receptor molecule can bind six to nine fibronectin molecules.

L7 ANSWER 33 OF 34 MEDLINE DUPLICATE 9
ACCESSION NUMBER: 86220122 MEDLINE
DOCUMENT NUMBER: 86220122 PubMed ID: 3519209
TITLE: Requirements for **substrate** recognition by bacterial leader peptidase.
AUTHOR: Dierstein R; Wickner W
SOURCE: EMBO JOURNAL, (1986 Feb) 5 (2) 427-31.

Journal code: 8208664. ISSN: 0261-4189.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198606
ENTRY DATE: Entered STN: 19900321
Last Updated on STN: 20000303
Entered Medline: 19860630

AB Many secreted and membrane proteins have amino-terminal leader peptides which are essential for their insertion across the membrane bilayer. These precursor proteins, whether from **prokaryotic** or eukaryotic sources, can be processed to their mature forms in vitro by **bacterial** leader peptidase. While different leader peptides have shared features, they do not share a unique sequence at the cleavage site. To examine the requirements for **substrate** recognition by leader peptidase, we have truncated M13 procoat, a membrane protein precursor, from both the amino- and carboxy-terminal ends with specific **proteases** or chemical cleavage agents. The fragments isolated from these reactions were assayed as **substrates** for leader peptidase. A 16 amino acid residue peptide which spans the leader peptidase cleavage site is accurately cleaved. Neither the basic amino-terminal region nor most of the hydrophobic central region of the leader peptide are essential for accurate cleavage.

L7 ANSWER 34 OF 34 WPIDS (C) 2002 THOMSON DERWENT
ACCESSION NUMBER: 1985-013953 [03] WPIDS
CROSS REFERENCE: 1987-328920 [47]; 1987-336315 [48]; 1987-355638 [51]; 1988-072542 [11]; 1990-068909 [10]; 1991-164187 [22]; 1992-365496 [44]; 1993-058202 [07]; 1994-176274 [21]; 1995-088790 [12]; 1995-088791 [12]; 1995-178127 [23]; 1997-392947 [36]; 1999-152092 [13]; 1999-526118 [44]
DOC. NO. NON-CPI: N1985-009950
DOC. NO. CPI: C1985-005668
TITLE: Procaryotic carbonyl hydrolase for use with surfactants - prepd. by cultivation of host transformed with recombinant vector.
DERWENT CLASS: D16 D25
INVENTOR(S): BOTT, R R; ESTELL, D A; FERRARI, E; HENNER, D J; WELLS, J A; STAHL, M L
PATENT ASSIGNEE(S): (GEMV) GENENCOR INT INC; (GETH) GENENTECH INC; (GEMV) GENENCOR INC
COUNTRY COUNT: 20
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
EP 130756	A	19850109	(198503)*	EN	79
R: AT BE CH DE FR GB IT LI LU NL SE					
AU 8429568	A	19850103	(198508)		
ZA 8404716	A	19841217	(198516)		
JP 60070075	A	19850420	(198522)		
DK 8403059	A	19850218	(198523)		
ES 8604646	A	19860701	(198638)		
ES 8608578	A	19861201	(198705)		
ES 8701839	A	19870301	(198715)		

US 4760025	A	19880726 (198832)	
AU 8937208	A	19891207 (199004)	
AU 8937149	A	19891123 (199011)	
EP 130756	B	19910206 (199106)	
R: AT BE CH DE FR GB IT LI LU NL SE			
DE 3484019	G	19910228 (199110)	
DE 3484079	G	19910314 (199112)	
AU 636109	B	19930422 (199323)	
DK 9300822	A	19930708 (199339)	
DK 9300823	A	19930708 (199339)	
US 5264366	A	19931123 (199348)	32
US 34606	E	19940510 (199418)	33
US 5310675	A	19940510 (199418)	39
NZ 236052	A	19940325 (199426)	
US 5346823	A	19940913 (199436)	39
JP 06315378	A	19941115 (199505)	35
JP 06319534	A	19941122 (199506)	35
US 5441882	A	19950815 (199538)	39
NZ 244586	A	19950828 (199540)	
JP 2594533	B2	19970326 (199717)	33
CA 1339893	C	19980602 (199833)	
JP 2889095	B2	19990510 (199924)	35
IE 81141	B	20000405 (200030)	
EP 130756	B2	20000628 (200035) EN	
R: AT BE CH DE FR GB IT LI LU NL SE			

APPLICATION DETAILS:

PATENT NO	KIND		APPLICATION	DATE
EP 130756	A		EP 1984-304252	19840622
ZA 8404716	A		ZA 1984-4716	19840621
JP 60070075	A		JP 1984-129928	19840622
ES 8604646	A		ES 1984-533645	19840622
ES 8608578	A		ES 1985-545148	19850712
ES 8701839	A		ES 1985-545147	19850712
US 4760025	A		US 1984-614612	19840529
AU 636109	B		AU 1989-37208	19890629
		Div ex	AU 1984-29568	
DK 9300822	A	Div ex	DK 1984-3059	19840622
			DK 1993-822	19930708
DK 9300823	A	Div ex	DK 1984-3059	19840622
			DK 1993-823	19930708
US 5264366	A	Cont of	US 1984-614615	19840529
		Cont of	US 1987-41885	19870423
			US 1991-797577	19911125
US 34606	E		US 1984-614612	19840529
			US 1990-556918	19900720
US 5310675	A	CIP of	US 1983-507419	19830624
		Cont of	US 1984-614616	19840529
		Cont of	US 1986-866389	19860522
		Cont of	US 1989-352326	19890515
			US 1991-805605	19911210
NZ 236052	A		NZ 1984-236052	19840621
US 5346823	A	Div ex	US 1984-614612	19840529
		Cont of	US 1987-91235	19870831
		Cont of	US 1990-521010	19900509
			US 1993-36592	19930324

JP 06315378	A	Div ex	JP 1984-129928	19840622
			JP 1993-244837	19840622
JP 06319534	A	Div ex	JP 1984-129928	19840622
			JP 1993-244823	19840622
US 5441882	A	Div ex	US 1984-614612	19840529
		Cont of	US 1987-91235	19870831
			US 1990-521010	19900509
NZ 244586	A		NZ 1984-244586	19840621
JP 2594533	B2		JP 1984-129928	19840622
CA 1339893	C		CA 1984-457209	19840622
JP 2889095	B2	Div ex	JP 1984-129928	19840622
			JP 1993-244823	19840622
IE 81141	B		IE 1984-1567	19840621
EP 130756	B2		EP 1984-304252	19840622
		Related to	EP 1987-200690	19840622
		Related to	EP 1989-202584	19840622
		Related to	EP 1987-200689	19860526

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 636109	B	Previous Publ.	AU 8937208
US 34606	E	Reissue of	US 4760025
NZ 236052	A	Div ex	NZ 208612
US 5346823	A	Div ex	US 4760025
US 5441882	A	Div ex	US 4760025
NZ 244586	A	Div ex	NZ 208612
JP 2594533	B2	Previous Publ.	JP 60070075
JP 2889095	B2	Previous Publ.	JP 06319534
EP 130756	B2	Related to	EP 246678
		Related to	EP 247647
		Related to	EP 357157

PRIORITY APPLN. INFO: US 1984-614617 19840529; US 1983-507419 19830624; US 1984-614491 19840529; US 1984-614612 19840529; US 1984-614615 19840529; US 1984-614616 19840529; US 1987-41885 19870423; US 1991-797577 19911125; US 1990-556918 19900720; US 1986-866389 19860522; US 1989-352326 19890515; US 1991-805605 19911210; US 1987-91235 19870831; US 1990-521010 19900509; US 1993-36592 19930324

AN 1985-013953 [03] WPIDS

CR 1987-328920 [47]; 1987-336315 [48]; 1987-355638 [51]; 1988-072542 [11]; 1990-068909 [10]; 1991-164187 [22]; 1992-365496 [44]; 1993-058202 [07]; 1994-176274 [21]; 1995-088790 [12]; 1995-088791 [12]; 1995-178127 [23]; 1997-392947 [36]; 1999-152092 [13]; 1999-526118 [44]

AB EP 130756 A UPAB: 20000725
Compsn. comprises a **procaryotic** carbonyl hydrolase, esp. a Bacillus hydrolase, and a host **micro-organism** transformed so as to be capable of expressing the hydrolase.

Compsn. comprising prepro-, pre- or pro-carbonyl hydrolase, esp. prosubtilisin, free from cells expressing the prepro-, pre- or pro-carbonyl hydrolase is also new. Liquid detergent compsn. comprising B amyloliquefaciens subtilisin is new.

Expression vector for a procaryotic carbonyl hydrolase comprising a DNA sequence encoding the hydrolase operable linked to a promoter compatible with a suitable host cell is also new.

USE/ADVANTAGE - Procaryotic carbonyl hydrolase is produced in recombinant host cells. The enzyme may be obtd. with various activity profiles differing from that of the precursor enzyme. The mutant enzymes are combined with surfactants or detergents in the usual way to give prods. for use in the laundry et

ABEQ EP 130756 B UPAB: 19930925

A process which comprises effecting a mutation in a Bacillus subtilisin enzyme or its pre- or prepoenzyme in one or more of the positions corresponding to Tyr-1, Asn+155, Tyr+104 Met+222, Gly+166, Gly+169, Glu+156, Ser+33, Phe+189, Tyr+217 and Ala+152 in B. amyloliquefaciens subtilisin or its pre- or preproenzyme, and testing for a desired activity change in the enzyme resulting from said mutation.

ABEQ US 4760025 A UPAB: 19930925

Cloned subtilisin gene with modifications at specific sites causes aminoacid substits. in the enzyme at positions 32, 155, 104, 222, 166, 64, 33, 169, 189, 217 or 157. The original subtilisin is obtd. from Bacillus amyloliquefaciens.

USE - The prods. are dispersed with one or more detergents (linear alkyl-benzenesulphonates, alkylphenyl sulphates, or higher sulphated alcohols or ethoxylated alcohols) for washing and cleaning compsns. for laundry use.

ABEQ US 5264366 A UPAB: 19940120

Isolated normally-sporulating mutant Bacillus produces no detectable proteolytic activity in a skim milk plate or casein assay during any phase of its growth, due to chromosomal deletions of 1 or more naturally-occurring codon specifying natural subtilisin **protease** or mature neutral **protease**.

Prepn. comprises (a) constructing (i) a plasmid which can integrate into Bacillus cDNA betwen the subtilisin gene and the in vitro-created deletion, and (ii) a plasmid which comprises a selectable marker and a deletion of the neutral **protease** gene; (b) transforming Bacilli with plasmid (i); (c) selecting transformants which can produce subtilisin; (c) selecting transformants contg. the marker; (d) selecting transformants from those in (c) whose subtilisin is inactive or deficient; (e) exiting the plasmid from the chromosomes of transformants obtd.; and (f) repeating steps (b)-(e).

ADVANTAGE - Deletions are made in any order, so the steps can be performed with either plasmid in any order.

Dwg. 0/16

ABEQ US 34606 E UPAB: 19940622

Subtilisin enzyme has a different amino acid at sites +32, +144, +104, +222, +166, +64, +33, +169, +189, +217 or +157 than that native to Bacillus amyloliquefaciens.

USE/ADVANTAGE - Improved pH activity profile, **substrate** specificity and oxidative stability.

Dwg.0/17

ABEQ US 5310675 A UPAB: 19940622

Recombinant expression vector comprises DNA encoding a subtilisin having a site-specific mutation at positions -1, +32, +155, +104, +222, +166, +64, +33, +169, +189, +217, +156, +221 or +152 in the corresp. sequence of mature subtilisin naturally produced by Bacillus amyloliquefaciens.

USE/ADVANTAGE - For producing prokaryotic carbonyl hydrolases

e.g. subtilisin and neutral **protease**.
Dwg.0/16
ABEQ US 5346823 A UPAB: 19941102
Prepn. of a mutant Baccillus subtilisin comprises obtaining DNA encoding subtilisin, substituting codons encoding Ser or Ala within a codon encoding Met, Trp, Cys or Lys, transforming a Bacillus cell with the mutated DNA, expressing the subtilisin and screening for improved oxidative stability.
ADVANTAGE - Increased oxidative stability.
Dwg.0/23
ABEQ US 5441882 A UPAB: 19950927
Prodn. of mutant subtilisin comprises mutation of a DNA fragment contg. a sequence that encodes the expression of modified bacillary subtilisin or its precursor in which one or more amino-acids are changed, e.g. Tyr(1), Asp(32), Asn(155), Tyr(104, Met(222), Gly(166), His(64), Ser(221), Ser(33), Phe(189), Tyr(217), Ala(152), Glu(156) and Gly(169), compared with subtilisin produced by Bacillus amyloliquefaciens; then transforming suitable bacillary host cells with the mutated DNA; propagation of the transformed cells; and recovery of the mutated subtilisin, which is then screened for alteration of enzyme characteristics, e.g. **substrate** specificity, oxidative stability, pH-activity profile and/or rate of formation of mature subtilisin from a precursor.
USE/ADVANTAGE - The prods. cleave proteins in a specific manner, providing more control of protein cleavage.
Dwg.0/16

FILE 'REGISTRY' ENTERED AT 14:17:51 ON 25 NOV 2002
L8 0 S NMLSEVERE | ACCDEYLQTKE | ADTVEPTGAKE/SQSP

Seq.

L9 1 S CATGCCATGGGTAGAACGGGCTGATACCCA/SQSN

L9 ANSWER 1 OF 1 REGISTRY COPYRIGHT 2002 ACS
RN 418487-11-3 REGISTRY
CN GenBank AX391153 (9CI) (CA INDEX NAME)
CI MAN
SQL 30

SEQ 1 catgccatgg gtagaacggg ctgataccca
========== ========== ==========
HITS AT: 1-30

FILE 'HCAPLUS' ENTERED AT 14:46:09 ON 25 NOV 2002)
L10 0 S L9

=> fil hom
FILE 'HOME' ENTERED AT 14:47:06 ON 25 NOV 2002